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THE UNIVERSITY OF ALBERTA

THE EFFECT OF CERTAIN HORMONES

ON ZINC METABOLISM

IN ADRENALECTOMIZED AND HYPOPHYSECTOMIZED RATS

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS

FOR THE DEGREE OF

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by

Kenneth Macey James, B.S.P., M.Sc.

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## ABSTRACT

The effects of adrenalectomy, hypophysectomy, and the administration of hormones on zinc concentration and  $\text{Zn}^{65}$  incorporation, were studied in a number of tissues of the male rat. The tissues studied were whole blood, serum, blood cells, liver, testis, adrenals, and the ventral and dorsolateral prostates. The hormones employed were desoxycorticosterone acetate, adrenocorticotrophic hormone, and growth hormone.

Adrenalectomy was found to cause a decrease in the  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate, a condition which was not changed by the administration of either desoxycorticosterone acetate or adrenocorticotrophic hormone. Desoxycorticosterone acetate, when administered over a period of fourteen days to intact and adrenalectomized rats, caused a decrease in the zinc concentration in whole blood. The action of desoxycorticosterone acetate on zinc metabolism was found to be significantly different in the tissues studied from that which has been reported previously for cortisone.

Adrenalectomy was shown to produce a marked decrease in the zinc concentration and  $\text{Zn}^{65}$  incorporation in the serum. Adrenocorticotrophic hormone was found to overcome the effect of adrenalectomy.





It is concluded that adrenocorticotrophic hormone has a controlling effect on the metabolism of zinc in the serum.

Liver from adrenalectomized animals which had received adrenocorticotrophic hormone showed an increased zinc concentration, suggesting a stimulatory action by the hormone on zinc in the liver. The daily administration of adrenocorticotrophic hormone to intact animals resulted in an increased  $\text{Zn}^{65}$  incorporation in the adrenal glands. The possibility of a relation between adrenal activity and zinc metabolism is postulated.

Hypophysectomy produced a decreased zinc concentration in the testis and dorsolateral prostate. Growth hormone administered to hypophysectomized animals, was found to maintain the zinc concentration in the testis, ventral and dorsolateral prostate, and to produce a significant increase in the zinc concentration in the adrenals. It is suggested that growth hormone plays a significant role in the maintenance of zinc balance in these tissues.

Several fractionation procedures were employed in an attempt to separate zinc-containing fractions from animal tissues. It was possible by chromatographic methods and radioautographic detection to show that a large portion of radioactive zinc in the dorsolateral prostate is in a combined form which is readily dialysable and thought to be associated with amino acids. The remainder of the zinc appears to be an integral part of a non-dialysable polypeptide or protein.



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## I. INTRODUCTION

Zinc, an element with widespread distribution in nature, is essential for the normal growth of both plants and animals. It comprises approximately 0.002 per cent of the human body and is, therefore, of the same order of magnitude as iron and copper (1). The early discovery of the metabolic importance of iron and copper was probably because of the ease of detection of the brightly colored iron and copper complexes. The white complexes of zinc are more difficult to distinguish. The importance of zinc to life is apparent when it is realized that zinc is an active component of the carbonic anhydrase molecule and of a number of other enzymes (2, 3).

There appears to be no doubt that there is a relationship between hormonal activity and zinc metabolism in the sex glands of the male rat. Mawson and Fischer (4) showed a very high concentration of zinc in the dorsolateral prostate. Gunn and Gould (5) also showed a high concentration and that there is a decreased  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate following castration. This effect could be corrected by the administration of testosterone. They have also reported that the removal of the pituitary results in a decreased capacity of the dorsolateral prostate to concentrate  $\text{Zn}^{65}$  and that replacement therapy, in the form of suitable doses of gonadotrophin or testosterone, could overcome this effect (6).



Work in this laboratory, reported by Rudzik and Riedel (7) showed that adrenalectomy resulted in a decreased incorporation of  $\text{Zn}^{65}$  in the dorsolateral prostate. The administration of cortisone was shown to cause a return to normal. These workers also showed that hypophysectomy had dramatic effects and that treatment with adrenocorticotrophic hormone (ACTH) caused significant changes in both hypophysectomized and intact animals (8). They concluded that it was possible to postulate a relationship between adrenal activity and zinc metabolism.

The work reported here was designed to evaluate further the relationship of hormone activity and zinc metabolism in tissues of the male rat. Also, it was thought desirable to attempt to fractionate the tissues, especially the dorsolateral prostate, into zinc-containing fractions. Such a fractionation might lead to an understanding of the significance of the large amounts of zinc found in the dorsolateral prostate.

Because of the results obtained from the cortisone study, it was thought important to investigate the relationship between zinc metabolism and a hormone of the adrenal cortex possessing a different physiological function from that of cortisone. A study was therefore made on the effect of adrenalectomy and the administration of desoxycorticosterone acetate (DCA) on zinc concentration and  $\text{Zn}^{65}$  incorporation in the same tissues as were previously examined.

A series of experiments was conducted on the effect of adrenalectomy and the administration of ACTH on zinc metabolism. Although Rudzik and Riedel (8) studied the effect of hypophysectomy and the administration of ACTH on zinc metabolism, it is difficult to say whether the results they obtained were due to a direct action of ACTH



or to an indirect action resulting from ACTH stimulation of the adrenals. It was felt that a series comparing adrenalectomy and the effect of ACTH would assist in answering this question.

A third series of experiments was conducted on the effect of hypophysectomy and the administration of growth hormone on zinc metabolism. This series was used to examine further the role of the pituitary in this area of zinc metabolism.

The three series thus studied were so planned as to allow comparisons between the results obtained in this thesis with the results of previous work reported by Rudzik and Riedel (7, 8).

From the results obtained in this study, it may be concluded that hormone effects on zinc metabolism are not only dramatic but are also significantly different. The action of DCA is quite different from that of cortisone. The action of ACTH is, to some extent at least, a result of the action of the hormone on the adrenals. It would appear that growth hormone plays a role in maintaining the zinc level in the testis, ventral and dorsolateral prostate, as well as having a stimulatory effect on the zinc level in the adrenals.

Following the administration of  $\text{Zn}^{65}$  to intact rats, fractionation of the dorsolateral prostate into radioactive fractions was accomplished by means of a chromatographic separation followed by radioautographic detection. The effect of dialysis and hydrolysis on the radioactive fractions was investigated.





## II. HISTORICAL

In 1869, Raulin (9), a pupil of Pasteur, demonstrated that zinc was necessary for the growth of Aspergillus niger, thus illustrating the role of zinc as an indispensable nutrient for this mold. In 1877, Lechartier and Bellamy (10) noted the presence of zinc in biological matter, and Raoult and Breton (11) reported zinc to be present in human liver. It was demonstrated, in 1904, that zinc is present in the liver and blood of the snail Sycotypus canaliculatus (12). Shortly after this finding, the first indication of a biological function for zinc was shown by the discovery that this element is an essential constituent of the respiratory pigment of Sycotypus (13). In 1911, Bertrand and Javillier (14) produced further evidence that zinc was necessary for the growth of Aspergillus niger.

Lutz (15), in 1926, published a paper in which he reviewed the literature accounts on the distribution of zinc, and in which he reported his findings on the occurrence of zinc in rat, cat, and man. He found that it was present in all the organs of the three different species of animals that he studied, and that the concentration of zinc in a specific tissue of a species is constant within a narrow range. In the literature review in his article, Lutz illustrated that zinc is a constituent of a great variety of plants and animals. Some examples of





plant life in which the occurrence of zinc was reported were, trees, molds, tubers, grains, yeast, oranges and fresh and dried vegetables. The occurrence of zinc in animal life included the following: marine organisms, reptiles, fowl and mammalian tissues and organs. The marine organisms included the conch, crayfish, sea cat, tunicate, oysters, sea lion, whale and certain fish. The animal tissues reported on comprised the blood, stomach, intestine, liver bile, kidney, pancreas, spleen, lung, thymus, thyroid, mammary gland, testis, brain, nervous tissue, voluntary muscle, heart, uterus, hair, hoof, and tumors. It is of interest to note that the above list does not include the prostate gland, but that in a paper by Drinker and Collier (16) the authors record part of a report published in 1921 by Bertrand and Vladesco (17) in which the statement is made that: "In man, the prostate is richer than the testicles; its content of zinc surpasses that of all the other organs of the body analyzed up to now . . . . .".

Although there is a great deal of doubt as to the accuracy of some of reported findings listed by Lutz (15), which were due to difficulties inherent in the methods used for the determination of zinc, nevertheless, the conclusion that zinc is a normal and seemingly universal constituent of living tissues, could be drawn.

In a paper by Drinker and Collier (16), entitled "The Significance of Zinc in the Living Organism", the authors presented a number of observations which to them suggested that zinc plays a significant part in the normal metabolism of plants and of animals. Among the observations cited were the universal occurrence of zinc in all types



of plant and animal life, and the uniformity of zinc concentration in the same tissues of different members of the same species. Further evidence which they presented to support their suggestion came from a variety of plant and animal experiments. This included the role of zinc in the development of Aspergillus niger, the chlorosis which develops when corn is deprived of zinc, the high zinc content of yolk of egg and milk, which represents the normal sources of nutriment for developing organisms during periods of extremely active growth, the high zinc content of the spermatic fluid, and the more rapid death of animals on a zinc free diet than those animals not so deprived.

During the 1920's, attempts were made to demonstrate an essential function for zinc in mammalian nutrition by feeding animals a diet deficient in zinc and comparing these animals with a similar group of animals fed on the same diet to which zinc had been added. Due to purification, the diets employed were so deficient in other essential nutrients, especially vitamins, that even with added zinc, the animals survived for only a short time (18, 19, 20). It was not until 1934 that Todd, Elvehjem and Hart (21), using specially prepared diets, produced conclusive evidence that zinc is essential for growth in rats. Further experimentation to substantiate their conclusions was reported in 1935 (22).

About this same time, Finch and Kinnison (23), reported a naturally occurring zinc deficiency in pecan trees growing in certain areas of Arizona. The disease produced by this deficiency was promptly alleviated by the administration of zinc salts. This discovery, along with the knowledge that a zinc deficiency produced



chlorosis in maize, was conclusive evidence to show that zinc plays an indispensable role in the growth of higher plants (24).

A major advance in the understanding of the biological function of zinc was made in 1939, when Keilin and Mann (2, 25) reported that zinc is an integral part of the carbonic anhydrase molecule. They were able to isolate and purify carbonic anhydrase, and to show that zinc comprises 0.33% of its molecule, and that zinc is essential to the mechanism of action of this enzyme.

In the past twenty years, other major strides in the study of zinc metabolism have been the discovery that swine parakeratosis is the result of a zinc deficiency (26), and that there are marked abnormalities in zinc metabolism in human postalcoholic cirrhosis (27, 28). The zinc concentration in the serum of patients with postalcoholic cirrhosis is markedly decreased and is significantly related to the severity of the disease.

#### A. ABSORPTION AND EXCRETION

The results of recent excretion studies made on rats during and following chronic feeding of  $Zn^{65}$  indicate that 50 to 60 per cent of the isotope is absorbed from the gastrointestinal tract (29). This does not agree with the findings of Feaster, Hansard, McCall and Davis (30) who, in 1955, reported zinc to be poorly absorbed from the gastrointestinal tract of the rat. They reported only 7.9 per cent of the ingested zinc was absorbed. It has been shown by studies on humans that zinc is excreted mainly through the intestines; the feces contains about 10 milligrams and the urine about 0.4 milligrams per day (28, 31, 32). It appears to make





little difference whether the zinc has been ingested or injected; the main route of elimination is by way of the feces. Work by Ballou (29), in 1960, showed that approximately 30 times as much absorbed  $Zn^{65}$  was excreted in the feces as in the urine. Fecal excretion of zinc increases following ingestion of zinc-rich foods, such as oysters (31).

Under normal conditions the amount of zinc found in the urine does not vary to any extent with the concentration of zinc in the food intake, neither does a high zinc level in the plasma, due to an injection of zinc, have any appreciable effect (32). Therefore it appears that the kidney in normal life takes little or no part in regulating the amount of zinc in the body. In this respect zinc resembles iron and differs from Na, K, Ca and Mg, all of which are excreted by the kidney, in amounts which vary with the requirements of the body and the amounts absorbed. It has been suggested that the small amount of zinc constantly found in the urine may represent the end product of some quite different, possibly metabolic, function of the kidney (32). In 1957, it was shown that zinc excretion is independent of urine volume (28). Millar, Fischer, Mawson and Elcoate (33) have shown that an intraperitoneal injection of Ca Di-Na EDTA greatly increases the urinary excretion of zinc.

The intestine as a regulating mechanism for ridding the body tissues of excess zinc does not have the same sensitivity as the kidney. To add to this lack of fine adjustment is the fact that the sensitivity of the regulating mechanism to a given dose varies with the individual and possibly with the degree of saturation of his storage organs (32). Support for this latter possibility arises from histochemical studies which showed increased quantities of zinc in the epithelium of the small intestine of the





rabbit following an intravenous injection of zinc acetate (34).

It has been shown that in dogs, a large proportion of injected  $\text{Zn}^{65}$  is eliminated by way of the external secretions of the pancreas (35). In a more recent publication it has been reported from a study of the excretion of  $\text{Zn}^{65}$  in the dog that there is more  $\text{Zn}^{65}$  secreted per volume collected in pure pancreatic juice than in bile or duodenal secretions (36). It was also noted that the radioactivity in the pancreatic juice appears earlier, reaches a higher peak, and is of longer duration than in corresponding collections of bile or duodenal juice. Measurements were made periodically during the first six hours following the intravenous injection of  $\text{Zn}^{65}$ .

One report in 1927 claimed to have definitely proved the skin to be a route of normal zinc excretion by demonstrating the presence of zinc in normal sweat (37). However, this has not been substantiated.

Working with preschool children, Scoular (38) concluded that 0.307 milligrams of zinc per kilogram of body weight per day is enough to supply the zinc needs of the human body in this age group. He also reported that from 0.04 to 6.0 per cent of the ingested zinc was found in the urine, and 42 to 164 per cent was excreted by the alimentary tract.

Stern, Nalder and Macy (39) reported an average daily urinary excretion of zinc in children to be 0.4 to 0.6 milligrams, while 7 to 12 milligrams was reported to be eliminated in the feces per day. In this study a daily intake of from 15.2 to 16.3 milligrams was reported, corresponding to a level of 0.4 to 0.6 milligrams per kilogram of body weight, and an average retention of 4.8 milligrams or 0.11 milligrams



per kilogram of body weight. The amount of zinc retained by the body following the ingestion of zinc was from 17.0 to 52.3 per cent while the apparent absorption was from 2.12 to 55.4 per cent.

A study of the zinc metabolism of young college women showed that the subjects excreted daily from 0.6 to 1.8 milligrams of zinc in the urine, and from 3.2 to 7.9 milligrams in the feces. The subjects ate self-selected diets that furnished from 9.8 to 14.4 milligrams of zinc daily, and this level was sufficient to maintain a positive zinc balance (40). This amount of zinc represents a normal intake, in that the usual human adult intake of zinc is 10 to 15 milligrams per day (32).

Sheline, Chaikoff, Jones and Montgomery (41) found that in mice over 50 per cent of an injected dose of  $\text{Zn}^{65}$  appeared in the feces in 170 hours while only 2 per cent appeared in the urine. In dogs, 25 per cent of the injected dose appeared in the feces after 12 to 14 days and 1.2 to 4.7 per cent in the urine.

Feaster et al (30) studying the placental transfer of  $\text{Zn}^{65}$  in the rat concluded that there is relatively free movement of the zinc, which has been absorbed and retained by the dam, across the placenta to the fetuses at all stages of gestation.

Zincuria, where an average of 2.1 milligrams of zinc per day was reported, was shown to accompany albuminuria (32). However, a direct relationship could not be shown between the amounts of albumin and zinc which were excreted. This would seem to be a contradiction to the hypothesis that in albuminuria the zinc is excreted bound to the protein. Zincuria of such magnitude as to be capable of depleting



body zinc stores, occurs in postalcoholic cirrhosis (28).

## B. ZINC DEFICIENCY IN ANIMALS

Zinc deficiency in the mouse manifests itself by a retardation of growth, decreased life span, and a loss of hair. Day (42), working with mice maintained on a diet very deficient in zinc, noted that the mice were retarded in growth from the first week of the experiment and that 50 per cent of the mice died within three to six weeks. The loss of hair was from the shoulders, back of the neck, and parts of the face. The zinc deficient mice also showed a decreased liver and kidney catalase activity.

Nishimura (43) concluded that colostrum has a much higher zinc content than later milk and he was able to show a number of disorders associated with a zinc deficiency in suckling mice deprived of colostrum. The disorders consisted of retarded growth and ossification, accelerated eruption of incisors and separation of eyelids, alterations of the dorsal skin, rosary-like tail, clubbed digits, and deformed nails. The oral administration of inorganic zinc prevented these disorders, while vitamin B<sub>1</sub>, certain vitamin B<sub>2</sub> compounds, liver pap and yeast had a mitigating effect.

It has been reported by Imada (44) that there is a synergistic interaction between zinc and vitamin B<sub>1</sub> in the intermediary metabolism of carbohydrates.

In 1934, Todd, Elvehjem and Hart (21) reported that rats fed on a diet low in zinc showed a markedly inferior growth rate compared to animals fed on the same diet with added zinc. It was also reported





that on a diet low in zinc, there was interference with the development of a normal fur coat in that there was a loss of hair about the neck and shoulders extending sometimes to involve the entire ventral surface.

The following year, Stirn et al (22), found that the growth rate of rats deficient in zinc was only one-third that of normal rats, and that zinc deficient animals required 52 per cent more ration to gain one gram in weight. The fur of the zinc-deficient rats softened and turned black to gray in six to seven weeks. Following the conclusion of the experiment, two animals fed adequate amounts of zinc resumed normal appearance.

In 1937, it was shown that rats fed on a diet containing 22 micrograms of zinc per day showed a 50 per cent decrease in growth rate, but that no zinc deficiency was present when animals received 40 micrograms of zinc per day. In the zinc-deficient rats there was a marked delay in absorption of foodstuffs through the intestinal mucosa, and it was thought that this delay was probably greater for nitrogenous products than for carbohydrates. Following the determination of urinary sugar, blood sugar, liver glycogen, and glucose tolerance, it was concluded that there were no disorders in the carbohydrate metabolism of zinc-deficient rats (45).

The activity of the pancreatic enzymes, amylase and proteinase, has been reported to be significantly lower in zinc-deficient rats and it could not be restored by the in vitro or in vivo addition of zinc (46). However, as mentioned by Vallee (3), there is a similar decreased enzyme activity in magnesium deficiency and this is considered to be the result of general debility.





Changes were found in the oesophagus, mouth and skin of rats placed on a restricted diet of two to four micrograms of zinc per day. In the oesophagus, and to a lesser extent in the mouth, there was a thickening of the epithelial cell layer together with the presence of an inner layer of incompletely keratinized cells. This change was said to be identical with the parakeratosis encountered in human skin lesions such as psoriasis. The alterations in the skin consisted of a hyperkeratinization, thickening of the epidermis, intra- and intercellular edema and loss of hair follicles with preservation of the sebaceous glands. The alopecia was most marked over the back of the rats (47).

Day and McCollum (48) noted retarded growth, eczema in two rats, and some alopecia in a group of rats fed on a diet very low in zinc. The alkaline phosphatase in blood serum was markedly reduced but it was not affected in bone and kidney. The activity of intestinal phosphatase (49) and carbonic anhydrase (50) has also been reported to be decreased by a deficiency of zinc. However, it has been claimed that carbonic anhydrase activity per unit of erythrocytes is unchanged (48).

The uric acid content of blood of zinc-deficient rats was found to be doubled on all diets tested (51). Carbohydrate, fat, protein, or nucleoprotein content of the diet did not influence the course of zinc deficiency and the hyperuricemia was not reversed until five weeks after the zinc had been added to the deficient diet. Since the uricase activity was completely normal, the hyperuricemia was thought to be due to increased uric acid synthesis and not lack of destruction.

It has been shown that in zinc-deficient rats there is an



atrophy of testicular germinal epithelium (52). Parizek (53, 54) has reported that the subcutaneous administration of cadmium salts to male rats leads to acute destruction of the testes. As the simultaneous administration of a large dose of zinc salts protects the testes against cadmium damage, it has been suggested that there is competitive inhibition between these two elements.

In 1953 (55), it was suggested that swine parakeratosis was a nutritional-deficiency disease. In 1955, Tucker and Salmon (26) deduced that this disease was the result of a zinc deficiency which is aggravated by a high concentration of calcium in the diet. Spontaneous parakeratosis, which is widespread, is characterized by dermatitis, diarrhea, vomiting, anorexia, severe weight loss, and eventual death. It has been observed that as the percentage of bone meal in the ration is increased, there is an increase in the incidence and severity of the disease (56).

Luecke, Hoefer, Brammell and Schmidt (57) observed a 100 per cent incidence of parakeratosis in 40 pigs receiving a ration containing 1.5 per cent calcium, 0.8 per cent phosphorus, and 31 parts per million of zinc. When the same ration was supplemented with 20 parts per million of zinc (as zinc carbonate) only one animal developed mild symptoms of the disease. From this same investigation, parakeratosis was produced in three of 10 pigs by feeding a ration containing 0.98 per cent calcium, 0.70 per cent phosphorus, and 29 parts per million of zinc. The disease produced in this manner was completely prevented by the supplementation of the ration with 20 parts per million of zinc.



In another study, it was shown that pigs on high calcium rations (1.1 - 1.4 per cent calcium) showed greatly decreased weight gain and an increased onset of parakeratosis when compared to pigs on a basal ration. The addition of 100 parts per million of zinc to the rations of pigs with established cases of parakeratosis produced immediate and dramatic responses in weight gain and elimination of skin lesions (56). The results obtained by adding 500 parts per million of zinc to the ration were no different from those obtained when 100 parts per million was added.

Lewis, Hoekstra and Grummer (58) have concluded that to control parakeratosis in swine, it is better to supplement the ration with zinc rather than restrict the calcium content. They have noticed no toxic effects in swine maintained on a diet containing 1000 parts per million of zinc.

It has been recommended that for growing pigs fed on diets containing up to one per cent calcium, the minimum zinc content of the diet for the prevention of parakeratosis is between 44 and 80 parts per million (59).

There appears to be no doubt that there is a relationship between zinc and calcium content of the diet and the incidence and severity of swine parakeratosis (26, 56, 57, 58, 59, 60, 61, 62).

A high level of calcium in the diet of swine does not interfere with the absorption of zinc. The supplementation of the diet with zinc increases the zinc concentration in the blood plasma, liver, and kidney but does not affect the zinc content of packed erythrocytes, spleen, intestine, or pancreas (63).





It has been suggested that zinc deficiency may be a factor in the beriberi syndrome. Working with tissues from Chinese subjects affected with acute, subacute and chronic beriberi, Eggleton (64, 65) found the zinc content of the toe nails, finger nails, and skin reduced to half the normal value and that there was a lowered zinc content of the blood. The reduced level of zinc in the blood was also noticed in cases of nutritional edema and semi-starvation.

### C. ZINC TOXICITY

As reported by Smith and Larson (66), rats fed on a diet containing one per cent zinc developed a severe microcytic and hypochromic anemia, growth was retarded and death may occur within six weeks. No reproduction occurred in rats feeding on a diet containing this level of zinc (67). Heart cytochrome oxidase activity was also reduced (68). The addition of 0.03 per cent copper to the diet prevented anemia, and increased cytochrome oxidase activity to above normal. The addition of copper to the zinc - rich diet resulted in significantly higher hemoglobin values, and the addition to the diet of a mixture of iron, copper, and cobalt supplements alone, had no effect (66). It has been reported that 0.1 milligrams of zinc per day fed together with iron assisted hemoglobin regeneration but that 0.5 milligrams of zinc retarded regeneration (69).

It has also been shown that at the level of one per cent zinc in the diet, zinc interfered with the development and mineralization of bones, and the fat content of the liver of rats was markedly reduced. This reduction in fat content is not due to faulty absorption of fat as the fecal fat content was normal (70, 71).





At the 0.7 per cent level of zinc in the diet of rats, the addition of copper prevented anemia but had no effect on the subnormal growth rate. The addition of a liver extract to the diet, however, produced a significant growth response (66). Folic acid had no effect (68).

Decreased rat liver catalase and cytochrome oxidase activities were recorded with a daily dietary level of 500 to 700 milligrams per cent zinc. The activity of both enzymes was restored to normal by the addition of 0.2 milligrams of copper per day. There appears to be a strong suggestion of an antagonistic effect between zinc and copper, although their actions may well be independent (72). As mentioned by Van Reen (72), copper and molybdenum are reciprocally antagonistic in animal metabolism, and it has been reported that molybdenum and zinc synergistically retard growth (73).

Other metabolic abnormalities noticed when rats were fed on a high zinc diet were an increase in urinary and fecal nitrogen, fecal phosphorous and sulphur, urinary uric acid, and creatinine. The phosphorous and sulphur content of the urine was concomitantly decreased (74).

Scott and Fisher (75), working with cats on a high zinc diet, observed marked fibrotic changes in the pancreas of all the cats under study.

In the dog, zinc gluconate at a dose of four milligrams per kilogram produced lassitude, decreased tendon reflexes, bloody enteritis, diarrhea, and paresis of the hind legs (76).

Ingestion of toxic amounts of zinc by humans produced symptoms that included malaise, dizziness, tightness of the throat, emesis, colic, and diarrhea. Inhalation of zinc oxide fumes resulted in fever,



malaise, depression, violent cough, excessive salivation, headache, and a high white blood cell count (3).

#### D. ZINC CONTENT OF ANIMAL TISSUES

Itallie (77) in 1907, noted that zinc is present in all tissues and organs of the body. The total zinc content of an adult fat-free body weighing 70 kilograms varies from 1.36 to 2.33 grams. The total iron content is 4.2 to 6.1 grams, and the copper content 112 to 231 milligrams (78).

On a wet weight basis, the amount of zinc present in vertebrate organs varies from 10 to 200 micrograms per gram. Most organs, including the pancreas, contain between 20 to 30 micrograms per gram. The pancreas does not have unusually large concentrations of zinc, although it has been shown that the islet tissue of fish is rich in the element, containing 100 to 1000 micrograms per gram. No significant difference has been detected in the zinc content of the pancreas in normal and diabetic patients (79). Liver, voluntary muscle, and bone contain about 40 to 60 micrograms of zinc per gram, while the endocrine organs and lymph nodes contain from 60 to 180 micrograms (80). Large quantities of zinc have been reported in certain tissues of the eye. This is especially true of the choroid and retina of perch and trout (81, 82, 83).

The zinc concentration of normal human whole blood is made up of the following parts: 22 per cent of the zinc is in the serum, three per cent in the leucocytes, and 75 per cent in the erythrocytes (84). The zinc content of whole blood varies from six to nine micrograms per milliliter of blood (84, 85, 86).

The individual leucocyte is extremely rich in zinc, containing



about 25 times the amount of zinc found in the erythrocyte, and probably contains more zinc than any other cell in the body (84, 85, 86). Hoch and Vallee (87) have reported that there is a definite zinc to protein ratio in human leucocytes and that this ratio is 82 to 117 micrograms of zinc per gram of protein. This study indicated that there are two types of complexes of zinc protein in leucocytes; one soluble and the other insoluble. The role of the zinc-containing protein of the leucocyte is unknown. Attempts have been made to study the leucocytic life span by tagging the cells with  $\text{Zn}^{65}$  but nothing conclusive has resulted (85, 88). It has been reported that the polymorphonuclear leucocyte contains essentially no zinc, and that leucocytic zinc is confined to the mononuclear cells (89).

The serum zinc concentration of a normal adult is from one to three micrograms per milliliter (84, 85, 90, 91, 92, 93, 94, 95). As zinc is not removed by clot formation, plasma and serum values are the same. The normal level of serum zinc in man does not vary with age or sex (94). From 62 to 66 per cent of the serum zinc is said to be firmly bound while the remainder is loosely bound (86, 94). Wolff (94) concluded that 20 to 30 per cent of serum zinc is associated with the albumins, 20 to 30 per cent with  $\alpha_1$  and  $\alpha_2$  globulin, and the remainder with  $\beta$  and  $\gamma$  globulin. He found that a large part of the firmly bound zinc was in the  $\alpha_1$  plus  $\alpha_2$  globulin fraction. When radioactive zinc was added to human serum in vitro and electrophoresis performed, two peaks of radioactivity were observed. The larger peak occurred in the slow moving part of the albumin and the smaller peak was in the region of the  $\alpha_2$  globulin (96). As a result of a study of the metal-combining globulin of





human plasma, it has been reported that zinc is bound to a  $\beta$  1 globulin at a neutral pH (97). It has been suggested that the iron-free portion of transferrin may be the means of transport for zinc in serum (86). Decreased serum zinc values have been observed in patients during the febrile stage of acute infectious diseases. The level rapidly returns to normal on recovery. Low serum zinc values have also been encountered in untreated cases of pernicious anemia and postalcoholic cirrhosis (27, 28, 86). Increases in serum zinc concentrations have been reported in hyperthyroidism, and essential hypertension, as well as following the administration of adrenaline, thyroxine, or thyrotropic hormone (94).

Smirnov (98) determined the zinc content of erythrocytes by a polarographic technique. He found that the amount of zinc, in micrograms per gram of erythrocytes was as follows: in man 13, rat 10, dog 9, rabbit 7.5, and in goose 6.5. Smirnov's value for the zinc content of human erythrocytes is in close agreement with values reported by other investigators (27, 91, 94). In the newborn infant, the erythrocyte zinc content is only 25 per cent of the normal adult value, increasing gradually until the adult value is reached at 12 years of age (99). It is also known that there is a reduced level of erythrocyte carbonic anhydrase in premature and newborn infants (100).  $\text{Zn}^{65}$  has been shown to penetrate the bovine erythrocyte readily, equilibrium between bovine serum and erythrocytes being achieved in six hours (101). It is thought that probably all the zinc present in the erythrocyte belongs to carbonic anhydrase (98, 102). In a study designed to show the relationship between carbonic anhydrase activity and zinc content of erythrocytes, good correlation was found between these two factors under normal conditions. In patients





with anemias, other than pernicious anemia, it was shown that both zinc and carbonic anhydrase levels were lowered in parallel fashion (103).

The prostate glands of the rabbit and of man, and the dorsolateral prostate of the rat, contain more zinc than any other soft tissue, with the exception of the leucocytes, and spermatozoa (4, 104, 105). Mawson and Fisher (104) reported an average of 874 micrograms of zinc per gram dry weight for the dorsolateral prostate of the rat, and 1,296 micrograms per gram for the rabbit prostate. Human prostates which they examined contained an average of 682 micrograms of zinc per gram dry weight. In 1951 (4), it was reported that on a wet weight basis, the dorsolateral prostate of the rat contained  $180.0 \pm 45.5$  micrograms of zinc per gram compared to  $13.7 \pm 3.2$  micrograms per gram for the ventral prostate. In 1958 (52), it was shown that in rats weighing between 258 and 279 grams, the mean zinc content of the dorsolateral prostate was between 111 and 116 micrograms of zinc per gram wet weight. Experiments with  $\text{Zn}^{65}$  indicated that the dorsolateral prostate of the rat concentrates the isotope from 10 to 25 times more than any other organ (106, 107). The removal of the dorsolateral prostate did not affect either fertility or fecundity in the rat (108). From a study conducted on the occurrence of  $\text{Zn}^{65}$  in the prostatic fluid of dogs following an intravenous injection, it was suggested that zinc is associated with an integral function of the prostate (109). It has been reported that the high concentration of zinc in rat dorsolateral prostate is accompanied by high carbonic anhydrase activity, although only part of the zinc in that tissue could be accounted for as carbonic anhydrase (110).



Only a small amount of carbonic anhydrase is found in human sperm, and little or none in the seminal plasma, although there is a high concentration of zinc in human semen. The highest concentration of zinc is found in the first fraction of the ejaculate, which is mainly composed of prostatic secretion. The amount of zinc present on a dry weight basis in human sperm is approximately 2000 micrograms per gram (105).

The turnover of radioactive zinc by various body tissues falls into three groups:-

(a) an initial rapid uptake followed by an exponential removal, as seen in the prostate, seminal vesicle, heart and lung.

(b) an initial rapid uptake with removal following two exponential functions as in the liver, kidney, spleen, ileum and pancreas.

(c) a slow initial uptake and a removal following a single exponential function as in the testis and femur (107).

#### E. ZINC METALLOENZYMES

Seven zinc metalloenzymes have been characterized to date. These are: the carbonic anhydrase of bovine erythrocytes (102), the alcohol dehydrogenase of yeast (111, 112) and of equine liver (113, 114), the glutamic dehydrogenase of bovine liver (115), rabbit muscle lactic dehydrogenase (116), carboxypeptidase (117, 118), and alkaline phosphatase (119).



(1) Carbonic Anhydrase

Mann and Keilin (120) reported that preparations of bovine erythrocyte carbonic anhydrase possessing a high degree of activity had a relatively high zinc content. In a subsequent report these authors (2) concluded that carbonic anhydrase is a zinc-protein compound. This conclusion was further confirmed by other investigators (50, 121, 122). The nature of the bonding between the zinc and the protein is unknown. The zinc is thought to be firmly bound as it is not removed by dialysis, even when the enzyme has become inactivated by long standing (102). The removal of the zinc from the carbonic anhydrase molecule results in an inactivation of the enzyme (2). Further evidence that zinc is an integral part of the enzyme molecule, and is firmly bound to the protein, was produced when it was shown that carbonic anhydrase in vitro will not exchange its zinc with zinc ions and simple zinc compounds (123). Zinc appears to be the only metal associated with this enzyme (102).

The zinc content of bovine erythrocyte carbonic anhydrase has been reported as 0.92 (124), 1.52 (102), and 1.38 (50) gram atoms per mole. This is based on a carbonic anhydrase molecular weight of 30,000 (125, 126). In human erythrocytes it has been calculated that there is 0.21 grams of carbonic anhydrase per 100 milliliters of packed red cells (80).

Carbonic anhydrase is strongly and reversibly inhibited by cyanide, sulphide, thiocyanate, azide, and British Anti-Lewisite (50, 127, 128). While these inhibitors are not specific for carbonic





anhydrase but inhibit other metalloproteins, the sulfonamides act as highly specific inhibitors (129). The inhibitory action of the sulfonamides is immediate, very strong, and completely reversible (129).

Davenport (130) found carbonic anhydrase present in the parietal cells of the gastric mucosa of cats and rats. The enzyme is also present in the prostate gland of rats, rabbits and humans (131), and in plants (132).

## (2) Alcohol Dehydrogenase of Yeast

By means of spectrographic and chemical analyses, Vallee and Hoch (111, 112) have shown that crystalline yeast alcohol dehydrogenase contains four atoms of zinc per molecule. The activity of the enzyme is directly dependent on zinc. The molecular weight of this enzyme is reported to be 150,000 (133). Any zinc present in excess of four atoms per molecule can be removed by dialysis against 0.1M phosphate buffer at pH6. This will also remove any other contaminating metals, the removal of which increases the enzymatic activity. At pH levels below six there is a loss of zinc and a concomitant decrease in enzyme activity. At pH 5.0, the enzymatic activity and zinc content are reduced to 50 per cent, and the enzyme is completely inactive and all of the zinc removed at pH 4.5 (112, 134).

A large number of chelating and complexing agents inhibit the enzymatic activity of this enzyme (112). It has been suggested from studies on the kinetics of competitive inhibition and on zinc-protein binding that the four molecules of diphosphopyridine nucleotide involved in yeast alcohol dehydrogenase activity are bound to the apoenzyme through zinc (111, 135, 136). It has also been suggested



that the zinc atom may be bound to the alcohol dehydrogenase molecule through a thiol bond (112).

(3) Alcohol Dehydrogenase of Horse Liver

The molecular weight of horse liver alcohol dehydrogenase has been reported as 73,000 (137) and 84,000 (138). This is approximately half of the molecular weight of yeast alcohol dehydrogenase. The horse liver enzyme contains two moles of zinc and binds two moles of diphosphopyridine nucleotide (113, 114, 139). The horse liver enzyme closely resembles the yeast enzyme in that it loses zinc and activity when dialysed at pH values below six (139).

(4) Glutamic Dehydrogenase of Bovine Liver

Glutamic dehydrogenase of beef liver is a zinc metallo-enzyme having a molecular weight of 1,000,000 (140) and containing from two to four gram atoms of zinc per mole of enzyme protein (115, 141, 142). The zinc which is an integral and functional component of this enzyme can be removed by dialysis at pH 4.5 (143). Inhibition of the enzyme can be caused by a number of reagents capable of coordinating zinc (141, 142). This inhibition is immediate but can be reversed by dilution or the addition of zinc ions to the reaction mixture (141).

(5) Rabbit Muscle Lactic Dehydrogenase

The molecular weight and diphosphopyridine nucleotide binding characteristics of this enzyme are not known. From chemical and spectrographical analysis it has been reported that the enzyme contains an average of 580 micrograms of zinc per gram of protein.



This enzyme can be inhibited by a number of metal-binding reagents, and some of the inhibitions can be prevented and reversed by zinc ions (116).

(6) Carboxypeptidase

Pancreatic carboxypeptidase has a molecular weight of 32,000 (144) and each enzyme molecule contains one atom of zinc (117, 118). Increasing amounts of zinc are released from carboxypeptidase as the pH is lowered and the activity of the enzyme is reduced at a similar rate (145). Inhibition of enzymatic activity by metal chelating agents such as 1,10-phenanthroline has been observed (117, 145).

(7) Alkaline Phosphatase

Mathies (119) has shown alkaline phosphatase from swine kidneys to be a zinc enzyme. He suggested that one molecule of the enzyme contains several atoms of zinc.

There are a number of enzymes which form zinc-enzyme complexes and whose activity is increased by the addition of zinc. However, zinc is usually not a specific requirement for these complexes in that the enzymes can be activated by other ions. These enzymes then are not true zinc metalloenzymes (3).

F. ZINC AND HORMONES

Whether or not zinc is an integral part of the insulin molecule has not been definitely established. Scott (146) and Fisher and Scott (147) crystallized insulin as the zinc salt and reported that insulin and zinc combined in a constant ratio. However, zinc is not required for either the





normal structure or function of the crystalline or amorphous insulin molecule. Preparations of zinc-free insulin and glucagon are completely active physiologically (3). Vallee (3) sums up the present status of zinc and the pancreatic hormones in the following statement: "There is a marked preponderance of hypotheses and speculations over precise data and crucial experimentation, but the role of zinc in the action of insulin and glucagon has not been settled with finality".

It has been reported that in the rat there is a marked augmentation in ovarian and uterine responses to pituitary extracts when zinc salts are added to the pituitary preparations (148, 149). It has also been noted that there is an increased gonadotropic action of follicle stimulating hormone when combined with zinc and copper salts. This augmented action attributed to zinc was found in both immature intact rats and hypophysectomized rats. The role of the zinc salts, it is thought, is to decrease the rate of absorption of the active material (150). Leatham (151), on the other hand, reported that a combination of zinc and equine gonadotropin, when administered in divided doses, had less ovarian stimulating activity than equine gonadotropin alone.

Holtermann, Heier, and Bergh (152) reported the presence of zinc in ACTH preparations obtained from whale pituitaries. The zinc content of their ACTH preparations was 197 parts per million. They suggested that zinc is an inherent and significant constituent of ACTH and that zinc is concentrated in the pituitary gland. Another group of investigators (153) made a study of the zinc content of various fractions of hog pituitary. They obtained a zinc value of 60 parts per million for the crude corticotropin fractions, and their results varied greatly for the





purified corticotropin fractions in that the values ranged from 124 to 390 parts per million of zinc.

From a study conducted on the binding of metal ions by ACTH it has been suggested that the hormone binds zinc and that there is a relationship between the extent of binding and the activity of the hormone (154).

Gunn and Gould (5) reported that the uptake of  $\text{Zn}^{65}$  by the dorsolateral prostate of the rat is influenced by both androgen and estrogen. The decrease in  $\text{Zn}^{65}$  uptake of the dorsolateral prostate as a result of castration was prevented by the administration of testosterone. On the other hand, the administration of estrogen either increased or decreased the  $\text{Zn}^{65}$  uptake of the dorsolateral prostate, depending on the dosage given.

Gunn and Gould (6) also found that the efficiency of the dorsolateral prostate of the rat to concentrate  $\text{Zn}^{65}$  was markedly depressed following hypophysectomy and that this inhibition could be prevented by administering suitable doses of chorionic gonadotropin or testosterone.

Millar, Elcoate and Mawson (155) reported that gonadotropins and testosterone propionate, when administered to immature rats, produced marked increases in the size, zinc concentration, and the rate of zinc uptake by the dorsolateral prostate.

Rudzik and Riedel (7) reported that the zinc concentration and  $\text{Zn}^{65}$  incorporation of the dorsolateral prostate of rats was decreased following adrenalectomy. They found that these effects of adrenalectomy could be overcome by treating the animals with cortisone for a period of 14 days. It was also reported that the zinc concentration of the adrenal



glands of intact rats was doubled by chronic administration of cortisone.

Rudzik and Riedel (8) have also found that hypophysectomy caused a marked decrease in the zinc concentration and  $\text{Zn}^{65}$  incorporation of the dorsolateral prostate of rats. Chronic treatment of hypophysectomized animals with ACTH resulted in a further decrease in the zinc concentration but an increase in  $\text{Zn}^{65}$  uptake of the gland. Chronic treatment of intact animals with ACTH caused a marked increase in both the zinc concentration and the  $\text{Zn}^{65}$  uptake. Also, it was shown from this study that the zinc concentration of the adrenal glands showed a marked increase 19 days following hypophysectomy, but a decreased  $\text{Zn}^{65}$  incorporation. The daily administration of ACTH to both intact and hypophysectomized animals showed a marked decrease in zinc concentration in the adrenals; whereas, the chronic treatment with ACTH caused an increase in the  $\text{Zn}^{65}$  incorporation of the adrenals in both intact and hypophysectomized animals.



### III. METHODS

#### A. ANIMALS

Throughout this investigation all animals used were male albino rats of the Sprague-Dawley strain.\* Control animals from the same lot of rats were kept under the same conditions in the same animal room. Normal intact animals were maintained on "Miracle" dog food pellets# and water ad libitum.

Adrenalectomy was performed by the dorsal route while the animals were under pentobarbital anesthesia. Adrenalectomized rats received one per cent sodium chloride solution ad libitum as a drinking solution along with dog food pellets.

Rats hypophysectomized by the transpharyngeal route were obtained from Hormone Assay Laboratories.& Macroscopic examination of the pituitary region showed no evidence of pituitary tissue remaining. Hypophysectomized rats were maintained on a diet of oranges, carrots, potatoes, brown bread, oatmeal, milk and water.

\*Northwest Rodent Co., Pullman, Washington.

#Ogilvie Flour Mills Co. Ltd.

&Hormone Assay Lab. Inc., Chicago, Ill.





Following the operation, adrenalectomized and hypophysectomized animals were observed over a 24 hour recovery period. One group was then administered a daily injection of hormone for fourteen days for the chronic experiments, a second group was maintained until the fourteenth day and then received a single dose of hormone for the acute experiments. A further group was retained as a control group.

## B. INJECTIONS

### (1) Desoxycorticosterone Acetate (DCA)

Desoxycorticosterone acetate\* (DCA) was prepared as a 0.5 per cent solution in sesame oil and was administered by intraperitoneal injection to both intact and adrenalectomized rats. The amount of DCA injected was 6 milligrams as a single dose (acute experiments) given 24 hours before killing, and 3 milligrams as a daily dose (chronic experiments) which was administered daily for a period of 14 days. The last dose was administered 24 hours before the animal was killed. The dosage levels of DCA were based on the work of other investigators (156, 157, 158).

### (2) Adrenocorticotrophic Hormone (ACTH)

ACTH (Acton "X", Nordic Biochemicals Ltd.) was administered by intraperitoneal injection to both intact and adrenalectomized

\*Supplied by Ciba Co. Ltd., Montreal.



rats. The amount of ACTH injected was 4 milligrams per 100 grams body weight as a single dose for the acute experiments and 0.4 milligrams per day for a period of 14 days for the chronic experiments. In all cases the last dose was given 24 hours before killing. The amounts of ACTH administered correspond to doses employed by Riedel, Logan, and Rossiter (159), and Rudzik and Riedel (8).

(3) Growth Hormone

Growth Hormone (bovine)\* was administered as an intraperitoneal injection to both intact and hypophysectomized rats. A dose of 0.2 milligrams was administered daily for a period of 14 days (chronic experiments). The amount of growth hormone which was injected was the same as that employed in a previous study conducted by Riedel (160).

(4) Radioactive Zinc

A neutralized solution of radioactive zinc ( $\text{Zn}^{65}$ ) # was injected intravenously via the external jugular vein while the animals were under pentobarbital anesthesia. The amount used was 70 microcuries per animal and was injected 24 hours before killing. The volume injected was never greater than 0.3 milliliters. The  $\text{Zn}^{65}$  was in the form of a  $\text{Zn Cl}_2$  solution and was a specially irradiated sample having a specific activity of approximately one millicurie per milligram of stable zinc. The 70 micrograms of stable zinc which was injected is an amount well

\*Supplied by National Institutes of Health, Bethesda, Md.

#Obtained from Dr. C. A. Mawson, Chalk River, Ont.

The amount of the injection was 4 milligrams per 100 grams of body weight and a single dose for the acute experiments and 10 milligrams per 100 grams of body weight for the chronic experiments.

Some of the rats for a period of 12 hours were kept in the dark and some in the light. The rats were kept in the dark for 12 hours and some in the light for 12 hours. The rats were kept in the dark for 12 hours and some in the light for 12 hours.

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below the 100 or 200 micrograms of zinc which was fed daily to weanling rats used as "diet controls" in the zinc studies of Millar, Elcoate, Fischer, and Mawson (161).

### C. TISSUE SAMPLES

At the appropriate time, all rats were killed by exsanguination while under pentobarbital anesthesia. A vertical incision was made up the abdomen to the diaphragm and a blood sample was withdrawn from the inferior vena cava into a heparinized hypodermic syringe fitted with a #18 needle.

A portion of the central lobe of the liver, the adrenals, one testis, and the whole prostate gland were removed, blotted to remove adhering blood, and cleaned of adipose tissue. The prostate was separated into the dorsolateral and ventral portions. All samples were placed in tared crucibles and weighed within five minutes of removal from the animal.

The samples were dried at 100° C. for four hours in a hot air drying oven and were then dry-ashed in a muffle furnace for not less than 12 hours at 600° C. Kagi and Vallee (162) have observed that the accuracy of the dry-ashing method is very dependent on the ashing temperature. Organic materials ashed at temperatures below 550° C. were shown to lose zinc by adsorption on carbon particles. Pijck, Hoste and Gillis (163) reported a loss of zinc when biological material was ashed at a temperature above 700° C. For these reasons the temperature of 600° C. was selected as being the optimum temperature.

The ash was dissolved by slowly boiling it with 2N HCl and





an estimation of the amount of zinc present was carried out.

#### D, QUANTITATIVE ESTIMATION OF ZINC

There are a number of methods which have been described for the determination of zinc in biological material. Fairhall (164) initially described three methods; gravimetric, turbidimetric and colorimetric. Of the various analytical methods since employed, colorimetric (165), polarographic (166) and emission spectrographic (167) methods are the ones most widely accepted. Neutron activation analysis has been recently employed and found to be suitable for the determination of small amounts of zinc in biological material (168). This method, of course, requires an atomic reactor.

The method selected for this investigation was the colorimetric method devised by Vallee and Gibson (169). It has been found by a number of workers to be highly reliable and has been used previously in this laboratory (7, 8, 86). The basis of this method is the production of a color by the reaction of zinc with the organic dye diphenylthiocarbazone (dithizone). At a pH of 5.5 and in the presence of a tartrate solution and complex-forming buffer, dithizone in carbon tetrachloride combines with zinc in stoichiometric proportions to form zinc dithizonate. This reaction is accompanied by a change in color from a bright green of the dithizone solution to a red of the zinc dithizonate complex. Other metals that may be present do not react with the dithizone under the conditions mentioned. At a pH of 5.5, the buffer containing potassium cyanide and sodium thiosulphate cause complex formation with copper, cobalt, nickel, bismuth, mercury, cadmium





and lead which may be present, while the sodium potassium tartrate solution causes complex formation with interfering iron and manganese (170).

A Beckman Model B spectrophotometer was used to determine the ratio of the amount of zinc dithizonate to dithizone at two critical wave lengths. These wave lengths were found to be 520 mμ and 595 mμ respectively. As shown in Figure 1, dithizone in carbon tetrachloride was found to have an absorption maximum at 595 mμ, a wave length at which the absorption of zinc dithizonate was minimal. Zinc dithizonate showed an absorption maximum at 520 mμ. Optical density values obtained at these two wave lengths were used in the formula derived by Vallee and Gibson (169) to calculate a corrected optical density factor.

$$X = \left( \frac{L_{520} - \frac{L_{595}}{R}}{\quad} \right) - \text{Blk.}$$

Where:

- X = corrected optical density of the sample
- L<sub>520</sub> = optical density of the sample at 520 mμ.
- L<sub>595</sub> = optical density of the sample at 595 mμ.
- R = ratio of optical density of dithizone in carbon tetrachloride at 595 mμ and 520 mμ.
- Blk = blank determination.

A standard zinc solution containing 10 micrograms of zinc and a blank were estimated with each set of determinations. Since zinc dithizonate was found to obey the Lambert-Beer Law (Figure 2) over the range 0-40 micrograms, the zinc concentration of the samples



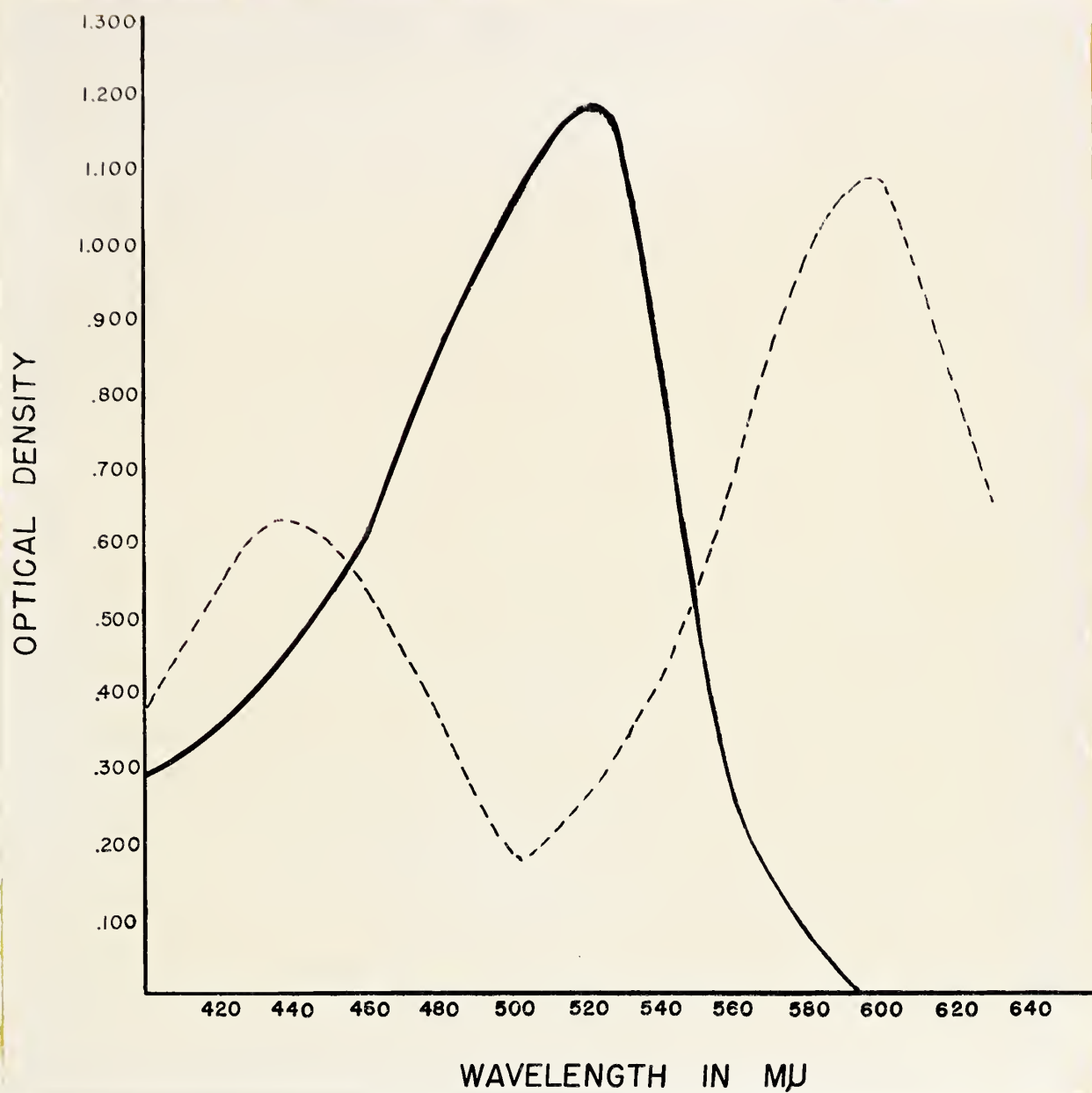


Figure 1.

Absorption curves of dithizone  
and  
dithizonate in carbon tetrachloride.

Dithizone \_\_\_\_\_

Zinc Dithizonate \_\_\_\_\_



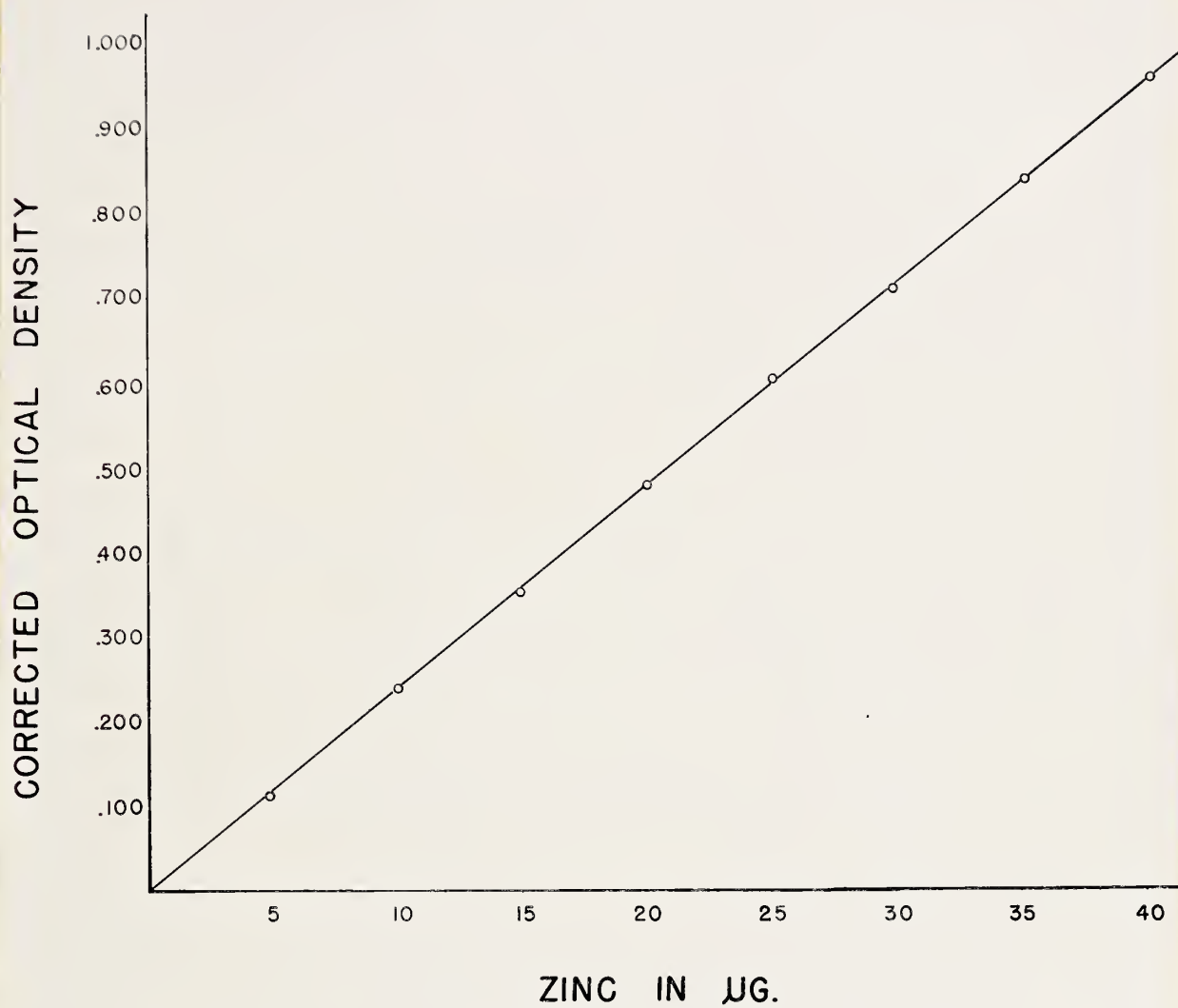


Figure 2.  
The relationship between  
corrected optical density  
and  
concentration of zinc.





could be determined by a direct comparison with the corrected optical density of the zinc standard.

The R value, which was determined each time an analysis was carried out, varied from 3.80 to 4.10. This value for R is in excellent agreement with the values published by Vikbladh (86).

#### E. DETERMINATION OF RADIOACTIVITY

Following the colorimetric determination, the zinc was converted to a water-soluble zinc salt by vigorously shaking the solution with five milliliters of 10 per cent  $H_2SO_4$ . The passage of the zinc into the aqueous phase was accompanied by a return of the carbon tetrachloride phase to the green color of dithizone. The aqueous layer was removed and the radioactivity present was determined by counting in a well scintillation counter with a NaI(Tl) crystal (Nuclear-Chicago) attached to a decade scaler (Tracerlab).

A  $Zn^{65}$  standard was counted with each set of samples. All counts were corrected for background, differences in the standard count, and for radioactive decay.

The standard counting error was determined. The standard error of a counting rate  $n_s - n_b$  is

$$\pm \sqrt{\left( \frac{\sqrt{N_s}}{t_s} \right)^2 + \left( \frac{\sqrt{N_b}}{t_b} \right)^2}$$



where:

$N_s$  = total sample count recorded

$t_s$  = total sample counting time

$N_b$  = total background count recorded

$t_b$  = total background counting time

$n_s = \frac{N_s}{t_s}$  = sample counting rate

$n_b = \frac{N_b}{t_b}$  = background counting rate

All samples were counted for three minutes and the lowest uncorrected count recorded was 2000 counts per minute. Background which was counted for three minutes was never higher than 500 counts per minute. Under these conditions the standard error the counting rate revealed was  $1500 \pm 28.9$  counts per minute. The percentage standard error was  $\pm 1.9$  per cent. As all of the samples had a net counting rate greater than 1500 counts per minute, the percentage standard error was never greater than 1.9 per cent.

#### F. REAGENTS AND GLASSWARE

In measurements of trace elements it becomes extremely important to avoid contamination. Distilled water was additionally purified by passing it through a Bantam dimineralizer (Barnstead Still and Sterilizer Co.) to remove all contaminating ions.

Analytical grade carbon tetrachloride was redistilled in an all Pyrex still.

Only analytical grade chemical reagents were used. The



ethylenediamine-tetraacetic acid method of Kaagi and Vallee (162) was used to remove contaminating metals from the certified reagent diphenylthiocarbazone (Fisher Scientific Co.). Following this the dithizone was further purified by the removal of the oxidation product diphenylthiocarbodiazone by the method of Milton and Waters (170). A concentrated solution containing 0.1 milligrams of dithizone per milliliter of carbon tetrachloride was stored in the dark at 4 - 6° C.

Both the buffer solution and the tartrate solution were shaken with a solution of dithizone in carbon tetrachloride until the dithizone remained green, to remove any contaminating zinc.

Pyrex glassware and porcelain crucibles were used throughout. All glassware was washed with soap and water, rinsed with distilled water, and then immersed in a bath of 2N HNO<sub>3</sub> for a period of not less than 12 hours. The glassware was rinsed with zinc-free water until free of acid as indicated by methyl red. Before use, the separatory funnels were rinsed with buffer solution and dithizone solution until a clear green color remained. In addition, flasks were rinsed with 0.01N NH<sub>4</sub>OH until yellow to methyl red. This ensured that the pH of any residue of rinse water was within the range of alkalinity which would not affect the extracted zinc dithizonate. Periodically all glassware was further cleaned by immersion in a chromic acid cleaning solution.

Porcelain crucibles were cleaned by boiling in aqua regia for at least 30 minutes, after which they were rinsed with zinc-free water.

Stop-cocks were greased with silicon grease.





## G. DEFINITION OF TERMS

Zinc concentration is expressed as micrograms of zinc per gram wet weight of tissue.

The specific activity of zinc in a tissue is defined as the counts per minute per microgram of zinc.

$$\text{Specific Activity} = \frac{\text{net sample counting rate corrected for decay}}{\mu\text{g. zinc in the sample}}$$

The term Relative Specific Activity is the ratio of the specific activity of the tissue relative to the specific activity of whole blood in the DCA series, and that of serum in the ACTH and Growth Hormone series. For convenience this figure is multiplied by  $10^2$ .

$$\text{Relative Specific Activity} = \frac{\text{specific activity of tissue}}{\text{specific activity of whole blood}} \times 10^2$$

Because of the greater complexity of the "mixed tissue" whole blood, serum was thought to provide a better basis for the calculation of relative specific activity. It is known that zinc enters the red blood cells where it is probably associated with the enzyme carbonic anhydrase. Thus, only labile or free zinc available to other tissues will be that present in the serum (102). Also, considerable difficulty was encountered in estimating the zinc concentration of whole blood samples as the color of the zinc dithizonate solution was found to fade in some samples before a colorimetric reading could be taken on the spectrophotometer. The reason for this is unknown and samples that showed this instability were discarded.

Corrected Specific Activity is obtained by multiplying the





specific activity of the blood or serum of a particular rat by the weight of the animal. The product is reduced by a factor of  $10^{-4}$  for convenience in handling. Since a constant amount of  $Zn^{65}$  was injected regardless of the size of the animal, it was necessary when blood or serum samples were to be compared, to correct the specific activity for differences in dilution.

Where a significant difference is shown by the letter "S" the differences are significant at 95 per cent confidence limits as determined by the formula (171):

$$\left( \frac{\quad}{X - X^1} \right) \pm t_{\alpha} \sqrt{\frac{(n - 1) S^2 + (n^1 - 1) S^{1^2}}{n + n^1 - 2}} \cdot \sqrt{\frac{1}{n} + \frac{1}{n^1}}$$

The ends of a bracket indicate the two values which are significantly different.



#### IV. RESULTS

Three series of experiments are presented. The first series was a study on the effect of adrenalectomy and the administration of DCA on the zinc concentration and  $\text{Zn}^{65}$  incorporation of selected tissues of the rat. The second series was a similar study on the effect of adrenalectomy and the administration of ACTH. The third series was an investigation on the effect of hypophysectomy and the administration of growth hormone on zinc metabolism. The results are summarized in the following sections. The individual results observed for each tissue are presented in table form in Appendices.

##### DESOXYCORTICOSTERONE ACETATE SERIES

A statistical evaluation and comparison of the results is presented for each tissue studied. The individual figures obtained for the tissues of each animal are presented in table form in Appendix A.

##### A. Whole Blood (Table I)

Table I presents a statistical comparison of the effects of adrenalectomy and the administration of DCA on the zinc concentration and  $\text{Zn}^{65}$  incorporation in whole blood.



TABLE I

Whole Blood - Effect of Adrenalectomy and the Administration of Desoxycorticosterone Acetate  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Groups of Animals	No. of Animals	Body Wt. in Gm.	Zinc Content µg/gm Wet Wt.	Sig.	Specific Activity	Corrected Specific Activity x 10 <sup>-4</sup>	Sig.
Intact	14	289	8.0 ± 1.9	} S	3903 ± 412	113.5 ± 25	
Intact + DCA (chronic)	6	262	5.0 ± 0.6		3530 ± 553	93.0 ± 19	
Intact + DCA (acute)	7	263	7.0 ± 1.9		3693 ± 952	100.0 ± 44	
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Adrenalectomized	15	286	7.9 ± 1.2	} S	4096 ± 765	117.3 ± 28	
Adrenalectomized + DCA (chronic)	5	258	6.3 ± 0.9		4093 ± 1227	103.7 ± 27	
Adrenalectomized + DCA (acute)	6	343	7.2 ± 1.5		3891 ± 962	133.6 ± 36	





The mean value obtained for the zinc concentration of whole blood in intact rats was found to be  $8.0 \pm 1.9$  which agrees very well with the average zinc concentration of normal human blood (8.1 micrograms per milliliter) as reported by Vikbladh (86).

The administration of DCA in the chronic experiments to both intact and adrenalectomized rats caused a significant decrease in the amount of zinc present in whole blood.

The corrected specific activity was found to be unchanged in any of the groups of animals.

#### B. Liver (Table II).

No changes were observed in the zinc concentration in liver in any of the groups of animals investigated. In agreement with the findings of Rudzik and Riedel (7), adrenalectomy appeared to have no effect on the zinc concentration or  $\text{Zn}^{65}$  incorporation in liver tissue. Intact animals which received DCA as either a single dose or as a daily dose showed a significant increase in the relative specific activity in liver tissue.

#### C. Testis (Table III)

No changes in either the zinc concentration or in the relative specific activity in the testis were observed in any of the groups of animals studied. As was found with liver, adrenalectomy had no effect on the zinc concentration or  $\text{Zn}^{65}$  incorporation in the testis.

#### D. Adrenals (Table IV)

The administration of DCA in both the acute and chronic experiments produced no significant changes in the zinc concentration or



TABLE II

Liver - Effect of Adrenalectomy and the Administration of Desoxycorticosterone Acetate  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Groups of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	33	47.7 ± 8.8		7309 ± 1878	169.8 ± 45	} S
Intact + DCA (chronic)	10	56.3 ± 13.6		7559 ± 1762	250.8 ± 42	
Intact + DCA (acute)	10	44.2 ± 4.9		7675 ± 2455	214.0 ± 29	
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Adrenalectomized	9	50.9 ± 8.4		8110 ± 642	175.8 ± 18	
Adrenalectomized + DCA (chronic)	12	52.6 ± 12.4		6444 ± 1053	174.5 ± 78	
Adrenalectomized + DCA	10	46.9 ± 6.4		7427 ± 1281	212.3 ± 54	



TABLE III

Testis - Effect of Adrenalectomy and the Administration of Desoxycorticosterone Acetate  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content µg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	33	29.9 ± 3.8		2379 ± 517	56.3 ± 13.4	
Intact + DCA (chronic)	10	28.9 ± 3.4		1864 ± 602	65.2 ± 15.7	
Intact + DCA (acute)	10	27.4 ± 2.2		2318 ± 368	67.0 ± 16.9	
<hr/>						
Adrenalectomized	9	27.0 ± 1.1		2762 ± 335	61.3 ± 7.3	
Adrenalectomized + DCA (chronic)	12	31.7 ± 4.7		2047 ± 229	55.5 ± 22.3	
Adrenalectomized + DCA (acute)	10	27.2 ± 3.2		2279 ± 515	69.3 ± 19.8	



TABLE IV

Adrenals - Effect of the Administration of Desoxycorticosterone Acetate on the Zinc

Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	33	30.9 ± 11.2		5184 ± 1735	110.8 ± 25	
Intact + DCA (chronic)	10	41.8 ± 20.9		4410 ± 460	124.7 ± 19	
Intact + DCA (acute)	10	38.2 ± 19.0		5112 ± 1516	130.6 ± 53	





Zn<sup>65</sup> incorporation in the adrenals.

E. Ventral Prostate (Table V)

Adrenalectomy caused no change in the zinc concentration or relative specific activity in the ventral prostate. The only significant difference in this tissue was recorded when intact animals received a single dose of DCA. This treatment resulted in an increased relative specific activity.

F. Dorsolateral Prostate (Table VI)

Adrenalectomy caused a significant decrease in the relative specific activity in the dorsolateral prostate. This result agrees with the published observations of Rudzik and Riedel (7). There was no significant differences in either the zinc concentration or relative specific activity in this tissue when the other groups of animals were compared.

DISCUSSION OF DCA SERIES

This work was a continuation of that previously reported by Rudzik and Riedel (7). It is therefore useful and desirable to compare the results recorded in this project using DCA with their results using cortisone. The procedures used in the previous series were the same as used here except that the animals were of Wistar strain in the cortisone experiments.

A. Whole Blood

The significant decrease observed in the zinc concentration in whole blood, in this work, when adrenalectomized rats received DCA



TABLE V

Ventral Prostate - Effect of Adrenalectomy and the Administration of Desoxycorticosterone Acetate  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	33	20.2 ± 5.8		6428 ± 998	149.1 ± 19.5	S
Intact + DCA (chronic)	10	20.4 ± 8.9		4068 ± 2658	155.8 ± 46.0	
Intact + DCA (acute)	10	16.7 ± 4.1		8216 ± 2240	212.5 ± 50.4	
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Adrenalectomized	9	17.6 ± 2.2		5954 ± 848	133.4 ± 24.5	
Adrenalectomized + DCA (chronic)	12	18.5 ± 3.3		5127 ± 623	138.1 ± 48.5	
Adrenalectomized + DCA (acute)	10	19.0 ± 5.8		6484 ± 1632	138.6 ± 27.3	



TABLE VI

Dorsolateral Prostate - Effect of Adrenalectomy and the Administration of Desoxycorticosterone Acetate  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content ug/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	33	112.7 ± 38.4		4222 ± 1173	122.9 ± 29.8	
Intact + DCA (chronic)	10	113.4 ± 43.0		3538 ± 1594	138.1 ± 18.5	
Intact + DCA (acute)	10	111.9 ± 45.6		4467 ± 1001	119.6 ± 13.2	
Adrenalectomized	9	105.0 ± 31.4		4399 ± 532	101.8 ± 19.3	
Adrenalectomized + DCA (chronic)	12	130.5 ± 49.7		3407 ± 951	106.1 ± 45.6	
Adrenalectomized + DCA (acute)	10	110.9 ± 17.4		4117 ± 1849	85.1 ± 21.7	

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in the chronic experiments, is directly opposite to that observed when cortisone was administered for a 14 day period. Intact animals that received DCA in the chronic experiments showed a lowered zinc concentration, whereas, the administration of cortisone to intact animals in similar experiments produced no change in the zinc concentration in whole blood.

When cortisone was administered to adrenalectomized animals either in acute or chronic experiments, the result was an increased  $\text{Zn}^{65}$  incorporation. This was not the case when DCA was administered to adrenalectomized animals as there was no change in the  $\text{Zn}^{65}$  incorporation. It would seem that these two hormones act in quite different ways on both zinc concentration and  $\text{Zn}^{65}$  incorporation, at least in this tissue.

#### B. Liver

The administration of DCA to intact animals in both the acute and chronic experiments resulted in a significant increase in the relative specific activity in the liver. However, the administration of cortisone to intact animals resulted in no change in the relative specific activity in liver.

Liver from adrenalectomized animals receiving DCA showed no changes in relative specific activity. On the other hand, adrenalectomized animals receiving cortisone showed an increased relative specific activity in liver.

It would appear that in the absence of the adrenal gland, and consequently in the absence of the gland's endogenous cortisone and DCA,



administered DCA has no effect on  $\text{Zn}^{65}$  uptake by the liver, but the administration of cortisone increases the tissue's ability to incorporate  $\text{Zn}^{65}$ .

As the administration of DCA to intact animals has the same effect on  $\text{Zn}^{65}$  incorporation as the administration of cortisone to adrenalectomized animals, this might suggest that in intact animals the end result of administering either DCA or cortisone is due to a mediating action on the part of the endogenous hormones.

#### C. Testis

The administration of DCA in both the acute and chronic experiments produced no changes in the relative specific activity in the testis. However, the administration of a single dose of cortisone to intact animals resulted in a decrease in the relative specific activity in this slowly metabolizing tissue.

#### D. Adrenals

Once again the difference is noted between administering cortisone and DCA, as DCA administered to intact animals caused no change in  $\text{Zn}^{65}$  incorporation, but cortisone given as a single dose to intact animals resulted in an increased  $\text{Zn}^{65}$  incorporation. There was no change in the zinc concentration in the adrenals following the administration of DCA to intact animals, but the administration of cortisone in the chronic experiments resulted in a significant increase in the zinc concentration in the tissue.



#### E. Ventral Prostate

Rudzik and Riedel (7) reported that adrenalectomized animals which had received cortisone in both acute and chronic experiments, showed an increased relative specific activity in the ventral prostate. When DCA was administered under the same conditions no change in relative specific activity resulted.

The result of administering a single dose of DCA to intact animals was an increased  $\text{Zn}^{65}$  incorporation, whereas, cortisone under the same conditions produced no change.

#### F. Dorsolateral Prostate

In both this study and the study conducted by Rudzik and Riedel (7), adrenalectomy caused a significant decrease in  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate. The daily administration of cortisone produced a marked increase in  $\text{Zn}^{65}$  incorporation, but this was not observed with DCA.

Such a difference in action, as observed in the results obtained by administering either DCA or cortisone, again suggests that the action of these two hormones on zinc metabolism is different if not directly opposite.

It may be suggested that a direct action of cortisone, but not DCA, is apparent on the  $\text{Zn}^{65}$  level in the tissues. The increased  $\text{Zn}^{65}$  level would be due to an increased uptake of  $\text{Zn}^{65}$  or a decreased elimination of the radioactive isotope.

The zinc concentrations which were determined for tissues





from intact animals in the DCA series compare favorably with those of the cortisone series with the exception of the adrenals and dorsolateral prostate. The reported zinc concentration of the dorsolateral prostate for intact animals of the DCA series is in good agreement with the value reported by Millar, Fischer, Elcoate, and Mawson (52) for animals of approximately the same weight. There is the possibility of a variation due to a difference in strain of rat in that the Wistar strain was used for the cortisone series and the Sprague-Dawley strain for the DCA series.

#### ADRENOCORTICOTROPIC HORMONE SERIES

A statistical evaluation and comparison of the results is presented for each tissue studied. The individual figures obtained for the tissues of each animal are presented in table form in Appendix B.

##### A. Serum (Table VII)

Table VII presents the results obtained after adrenalectomy and the administration of ACTH on the zinc concentration and  $Zn^{65}$  incorporation in serum.

Adrenalectomy caused a significant decrease in the zinc concentration and in the corrected specific activity in the serum. ACTH administered in both the acute and chronic experiments to adrenalectomized rats resulted in a return to normal of the zinc concentration in the serum. The administration of ACTH to adrenalectomized rats also significantly increased the corrected specific activity in the serum.





TABLE VII

Serum - Effect of Adrenalectomy and the Administration of ACTH on the

Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Body Wt. in Gm.	Zinc Content $\mu\text{g/gm Wet Wt.}$	Sig.	Specific Activity	Corrected Specific Activity $\times 10^{-4}$	Sig.
Intact	14	283	1.0 $\pm$ 0.39	S	3443 $\pm$ 613	97.7 $\pm$ 22.6	S
Intact + ACTH (chronic)	14	290	1.0 $\pm$ 0.3		3240 $\pm$ 401	94.3 $\pm$ 13.1	
Intact + ACTH (acute)	13	275	0.9 $\pm$ 0.39		3198 $\pm$ 510	87.9 $\pm$ 14.2	
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Adrenalectomized	15	289	0.5 $\pm$ 0.14	S	2216 $\pm$ 490	63.7 $\pm$ 14.0	S
Adrenalectomized + ACTH (chronic)	14	276	1.0 $\pm$ 0.43		4624 $\pm$ 1154	127.0 $\pm$ 31.5	
Adrenalectomized + ACTH (acute)	14	287	1.16 $\pm$ 0.57		6360 $\pm$ 1485	197.3 $\pm$ 67.1	



B. Cells (Table VIII)

Adrenalectomy caused no change in the zinc concentration of the cells. However, it did cause a marked increase in the relative specific activity. The administration of ACTH to adrenalectomized animals in both the acute and chronic experiments resulted in a significant decrease in the relative specific activity bringing the value down to approximately the value for intact animals. The administration of ACTH to intact animals in the chronic experiments resulted in an increased relative specific activity.

C. Liver (Table IX)

Adrenalectomy caused a significant increase in the relative specific activity in the liver. The administration of ACTH to adrenalectomized animals caused a significant decrease in the relative specific activity.

Intact animals that had received ACTH in both the acute and chronic experiments showed a significant increase in the relative specific activity.

The adrenalectomized rats that received ACTH in both the acute and chronic experiments showed an increased zinc concentration in the liver.

D. Testis (Table X)

Adrenalectomy caused a significant increase in the relative specific activity in the testis. The administration of ACTH to



TABLE VIII

Cells - Effect of Adrenalectomy and the Administration of ACTH on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	7.6 ± 2.1		1685 ± 205	51.9 ± 12.3	} S
Intact + ACTH (chronic)	14	7.7 ± 0.82		1923 ± 260	66.4 ± 10.9	
Intact + ACTH (acute)	13	6.3 ± 1.36		1810 ± 213	58.7 ± 12.4	
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Adrenalectomized	15	7.5 ± 1.2		1983 ± 395	92.7 ± 22.2	} S
Adrenalectomized + ACTH (chronic)	14	8.0 ± 1.6		2255 ± 735	60.3 ± 16.3	
Adrenalectomized + ACTH (acute)	14	8.1 ± 1.8		2857 ± 362	44.9 ± 12.1	

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TABLE IX

Liver - Effect of Adrenalectomy and the Administration of ACTH  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	49.6 ± 6.0	}	3095 ± 276	93.0 ± 16.1	}
Intact + ACTH (chronic)	14	46.3 ± 5.1		3237 ± 405	108.2 ± 14.3	
Intact + ACTH (acute)	13	48.4 ± 4.6		4196 ± 450	133.8 ± 17.1	
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Adrenalectomized	15	46.2 ± 5.7	}	3362 ± 1076	157.9 ± 53.9	}
Adrenalectomized + ACTH (chronic)	14	53.2 ± 8.8		4065 ± 1149	105.9 ± 46.9	
Adrenalectomized + ACTH (acute)	14	60.9 ± 8.9		5436 ± 475	87.9 ± 19.9	

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TABLE X

Testis - Effect of Adrenalectomy and the Administration of ACTH on the Zinc

Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	29.7 ± 1.6		1125 ± 88	33.5 ± 6.8	S
Intact + ACTH (chronic)	14	28.7 ± 2.5		998 ± 158	34.5 ± 4.5	
Intact + ACTH (acute)	13	30.1 ± 1.1		1138 ± 123	37.3 ± 8.5	
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Adrenalectomized	15	30.3 ± 0.7		1136 ± 32	53.9 ± 13.6	S
Adrenalectomized + ACTH (chronic)	14	29.4 ± 1.8		1569 ± 269	37.0 ± 9.0	
Adrenalectomized + ACTH (acute)	14	30.0 ± 3.5		1858 ± 331	29.6 ± 10.4	



adrenalectomized rats in both the acute and chronic experiments resulted in a marked decrease in the relative specific activity in the testis.

None of the treatments used caused any change in the zinc concentration in the testis.

#### E. Adrenals (Table XI)

Intact animals that received ACTH in the chronic experiments showed a significant increase in the relative specific activity in the adrenals with no change in the zinc concentration.

Intact animals that received ACTH in the acute experiments showed an increased zinc concentration in the adrenals, but no change in the relative specific activity.

#### F. Ventral Prostate (Table XII)

ACTH injected into intact or adrenalectomized animals produced no changes in the zinc concentration in the ventral prostate.

Adrenalectomy caused an increased relative specific activity which was decreased significantly by the administration of ACTH in both the acute and chronic experiments.

The administration of ACTH to intact animals in the chronic experiments resulted in an increased relative specific activity in this tissue.

#### G. Dorsolateral Prostate (Table XIII)

The administration of ACTH to adrenalectomized animals in both the acute and chronic experiments resulted in a significant decrease



TABLE XI

Adrenals - Effect of the Administration of ACTH on the Zinc Concentration  
and  $\text{Zn}^{65}$  Incorporation

Group of Animals	No. of Animals	Zinc Content $\mu\text{g/gm}$ Wet Wt.	Sig.	Specific Activity	Relative Specific Activity $\times 10^2$	Sig.
Intact	8	25.3 $\pm$ 4.8	S	2983 $\pm$ 611	88.4 $\pm$ 19.7	S
Intact + ACTH (chronic)	14	24.7 $\pm$ 6.5		3418 $\pm$ 948	119.6 $\pm$ 24.6	
Intact + ACTH	13	36.8 $\pm$ 5.3		2707 $\pm$ 486	87.0 $\pm$ 16.9	





TABLE XII

Ventral Prostate - Effect of Adrenalectomy and the Administration of ACTH  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	18.7 ± 3.0		3468 ± 304	102.4 ± 22.6	} S
Intact + ACTH (chronic)	14	16.2 ± 1.8		3773 ± 318	127.2 ± 14.1	
Intact + ACTH (acute)	13	17.0 ± 1.9		3599 ± 242	116.0 ± 20.9	
<hr style="border-top: 1px dashed black;"/>						
Adrenalectomized	15	16.8 ± 2.0		2671 ± 439	141.6 ± 30.9	} S
Adrenalectomized + ACTH (chronic)	14	16.2 ± 2.9		4815 ± 941	110.8 ± 23.7	
Adrenalectomized + ACTH (acute)	14	18.7 ± 6.0		5362 ± 757	83.5 ± 8.3	



TABLE XIII

Dorsolateral Prostate - Effect of Adrenalectomy and the Administration of ACTH on  
the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	µg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	123.9 ± 34.9		2351 ± 416	70.2 ± 19.6	
Intact + ACTH (chronic)	14	120.9 ± 30.2		2511 ± 259	83.8 ± 13.7	
Intact + ACTH	13	126.0 ± 32.8		2311 ± 526	73.8 ± 15.2	
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Adrenalectomized	15	136.9 ± 43.2		1823 ± 332	86.8 ± 26.4	} S
Adrenalectomized + ACTH (chronic)	14	129.1 ± 36.1		3026 ± 798	68.9 ± 15.4	
Adrenalectomized + ACTH (acute)	14	169.0 ± 74.7		3773 ± 859	60.9 ± 21.3	



in the relative activity in the dorsolateral prostate.

No changes occurred in the zinc concentration in the dorsolateral prostate as a result of any of the treatments used.

#### DISCUSSION OF ACTH SERIES

As adrenalectomy caused a decrease in the zinc concentration and also in the corrected specific activity in the serum, it would appear that in the absence of the adrenal glands, zinc is withdrawn or lost from the serum to a greater extent than when the adrenal glands are present. The administration of ACTH to adrenalectomized animals in both the acute and chronic experiments caused the zinc concentration to be returned to normal and caused a significant increase in the  $\text{Zn}^{65}$  incorporation in the serum. It would appear that the administration of ACTH to adrenalectomized animals has corrected the lesion brought about by the removal of the adrenal glands.

With the exception of the serum and dorsolateral prostate, adrenalectomy resulted in an increased relative specific activity in each of the tissues studied. As relative specific activity is based on the specific activity of the serum, and as the corrected specific activity of the serum is low in adrenalectomized animals, this would indicate that the tissues are not necessarily taking up more  $\text{Zn}^{65}$  but that the serum  $\text{Zn}^{65}$  is being more rapidly eliminated than occurs in intact animals.

As the administration of ACTH to adrenalectomized animals, in both the acute and chronic experiments, caused an increased corrected specific activity in the serum, it follows that ACTH administered to adrenalectomized animals would result in a decreased relative specific





activity in the other tissues. This relationship was observed for all of the tissues studied.

In the dorsolateral prostate no significant difference was found between the relative specific activities in the tissue from intact and adrenalectomized animals, even though the corrected specific activity in the serum was low in adrenalectomized animals. This would suggest a decreased  $\text{Zn}^{65}$  incorporation by the dorsolateral prostate or an increased loss of the radioactive isotope from this tissue in adrenalectomized animals.

In the cells, liver, adrenals and ventral prostate, a significant increase in relative specific activities was observed when ACTH was administered daily for 14 days to intact animals. This was also observed in liver tissue when ACTH was given as a single dose. As none of the tissues from intact animals that received ACTH in the chronic experiments showed any changes in zinc concentration and as no changes were observed in the corrected specific activities in serum samples in this group of animals, it would appear that the increased relative specific activity was due to an increased incorporation of  $\text{Zn}^{65}$ .

Liver and adrenals also showed changes in zinc concentration. Liver from adrenalectomized animals that had received ACTH in both the acute and chronic experiments showed a significant increase in zinc concentration. This trend is the same as was found in the zinc concentration in serum, although adrenalectomy did not cause a significant decrease in the zinc concentration in the liver. As ACTH administered in the absence of the adrenals caused an increased zinc concentration in the liver, it would appear that ACTH has a direct stimulatory action on zinc metabolism in the liver. The adrenal glands of intact animals that



received a single dose of ACTH showed an increased zinc concentration. This was not observed when ACTH was administered for a period of 14 days.

### GROWTH HORMONE SERIES

A statistical evaluation and comparison of the results is presented for each tissue studied. The individual figures obtained for the tissues of each animal are presented in table form in Appendix C.

#### A. Serum (Table XIV)

The administration of growth hormone to intact animals resulted in a significant increase in the zinc concentration in the serum. Hypophysectomy did not affect the zinc concentration in the serum nor did the subsequent administration of growth hormone to hypophysectomized animals.

Hypophysectomy resulted in a decreased corrected specific activity in the serum, a situation which was not changed by the administration of growth hormone.

#### B. Cells (Table XV)

Hypophysectomy caused a significant increase in the zinc concentration and relative specific activity in the cells. No other changes were observed.

#### C. Liver (Table XVI)

Hypophysectomy caused an increased zinc concentration and



TABLE XIV

Serum - Effect of Hypophysectomy and the Administration of Growth Hormone on the Zinc

Concentration and Zn <sup>65</sup> Incorporation						
Group of Animals	No. of Animals	Body Wt. in Gms.	Zinc Content, $\mu\text{g/gm Wet Wt.}$	Sig.	Specific Activity	Corrected Specific Activity $\times 10^{-4}$ Sig.
Intact	14	283	1.0 $\pm$ 0.39	S	3443 $\pm$ 613	97.7 $\pm$ 22.6
Intact + Growth Hormone (chronic)	16	349	1.4 $\pm$ 0.37		2674 $\pm$ 460	93.0 $\pm$ 13.7
<hr/>						
Hypophysectomized	14	173	1.1 $\pm$ 0.41		3087 $\pm$ 784	53.9 $\pm$ 16.3
Hypophysectomized + Growth Hormone (chronic)	16	220	1.1 $\pm$ 0.44		2720 $\pm$ 528	59.4 $\pm$ 11.4



TABLE XV

Cells - Effect of Hypophysectomy and the Administration of Growth Hormone on the

Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	7.6 ± 2.1	}	1685 ± 205	51.9 ± 12.3	}
Intact + Growth Hormone (chronic)	16	7.2 ± 1.4		1574 ± 269	60.2 ± 15.8	
Hypophysectomized	14	9.5 ± 1.6	}	2193 ± 336	74.8 ± 19.2	}
Hypophysectomized + Growth Hormone (chronic)	16	9.0 ± 1.9		1752 ± 356	67.4 ± 15.3	





TABLE XVI

Liver - Effect of Hypophysectomy and the Administration of Growth Hormone on  
the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	49.6 ± 6.0	}	3095 ± 276	93.0 ± 16.1	}
Intact + Growth Hormone (chronic)	16	46.6 ± 5.1		3058 ± 256	115.3 ± 16.6	
<hr/>						
Hypophysectomized	14	58.4 ± 11.5	}	4782 ± 658	170.0 ± 40.2	}
Hypophysectomized + Growth Hormone (chronic)	16	49.2 ± 6.6		3618 ± 609	139.9 ± 29.6	



relative specific activity in the liver. The administration of growth hormone to hypophysectomized rats caused a significant decrease in the zinc concentration returning it to normal and a decrease in the relative specific activity.

The administration of growth hormone to intact animals resulted in an increased relative specific activity.

#### D. Testis (Table XVII)

Hypophysectomy caused a significant decrease in the zinc concentration in the testis and an increased relative specific activity.

The administration of growth hormone to either intact or hypophysectomized animals had no effect on the zinc concentration or relative specific activity in the testis.

#### E. Adrenals (Table XVIII)

The only effect of hypophysectomy observed in the adrenals was an increased zinc concentration.

The administration of growth hormone to hypophysectomized animals resulted in a significant decrease in the relative specific activity and a significant increase in zinc concentration.

#### F. Ventral Prostate (Table XIX)

Hypophysectomy resulted in a significant increase in the zinc concentration in the ventral prostate and a marked decrease in the relative specific activity.



TABLE XVII

Testis - Effect of Hypophysectomy and the Administration of Growth Hormone on the

Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	29.7 ± 1.6	S	1125 ± 88	33.5 ± 6.8	S
Intact + Growth Hormone (chronic)	16	31.3 ± 1.2		952 ± 85	36.7 ± 5.3	
Hypophysectomized	14	25.5 ± 1.8	S	1603 ± 279	56.3 ± 15.0	S
Hypophysectomized + Growth Hormone (chronic)	16	24.7 ± 1.8		1440 ± 309	53.7 ± 16.1	





TABLE XVIII

Adrenals - Effect of Hypophysectomy and the Administration of Growth Hormone  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	8	25.3 ± 4.8	S	2983 ± 611	88.4 ± 19.7	- 73 -
Intact + Growth Hormone (chronic)	16	28.8 ± 4.8		2708 ± 119	102.1 ± 22.1	
Hypophysectomized	14	45.0 ± 12.8	S	2843 ± 568	95.6 ± 19.6	S
Hypophysectomized + Growth Hormone (chronic)	16	58.2 ± 8.7		1427 ± 432	73.6 ± 8.0	



TABLE XIX

Ventral Prostate - Effect of Hypophysectomy and the Administration of Growth Hormone  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	18.7 ± 3.0	S	3468 ± 304	102.4 ± 22.6	S
Intact + Growth Hormone (chronic)	16	17.0 ± 4.5		3025 ± 515	116.4 ± 26.8	
Hypophysectomized	14	50.2 ± 14.1	S	1811 ± 695	57.8 ± 18.7	S
Hypophysectomized + Growth Hormone (chronic)	16	54.1 ± 18.1		1298 ± 596	60.3 ± 9.9	



#### G. Dorsolateral Prostate (Table XX)

Hypophysectomy resulted in a significant decrease in the zinc concentration in the dorsolateral prostate.

The administration of growth hormone to intact animals resulted in a significant increase in zinc concentration in this tissue.

No significant changes were observed in relative specific activity with any of the treatments used.

#### DISCUSSION OF GROWTH HORMONE SERIES

Hypophysectomy, unlike adrenalectomy, caused no change in the zinc concentration in the serum. However, hypophysectomy did cause a significant increase in the zinc concentration in the cells, liver, adrenals and ventral prostate. Rudzik and Riedel (8), using the same strain of animal and the same procedures, reported a similar significant increase in zinc concentration of hypophysectomized animals in the liver and adrenals but they reported no change in the ventral prostate. The decreased zinc concentration reported above in the testis and dorsolateral prostate of hypophysectomized animals agreed in part with the published report of Rudzik and Riedel (8). They reported that hypophysectomy caused a decreased zinc concentration in the dorsolateral prostate but no change in the testis.

A cessation of growth, as indicated by animal body weight, was apparent in hypophysectomized animals (Table XXI). Also, the typical atrophy associated with hypophysectomy was observed in the gonads, accessory sex organs and adrenals. It has been reported that



TABLE XX

Dorsolateral Prostate - Effect of Hypophysectomy and the Administration of Growth Hormone  
on the Zinc Concentration and Zn<sup>65</sup> Incorporation

Group of Animals	No. of Animals	Zinc Content μg/gm Wet Wt.	Sig.	Specific Activity	Relative Specific Activity x 10 <sup>2</sup>	Sig.
Intact	14	123.9 ± 34.9	} S	2351 ± 416	70.2 ± 19.6	
Intact + Growth Hormone (chronic)	16	159.8 ± 35.1		1678 ± 294	64.3 ± 14.3	
<hr/>						
Hypophysectomized	14	61.2 ± 23.2	} S	1963 ± 512	65.1 ± 15.7	
Hypophysectomized + Growth Hormone (chronic)	16	55.8 ± 13.7		1932 ± 544	80.3 ± 22.7	





TABLE XXI

The Effect on Tissue Weight of Administration  
of Growth Hormone to Hypophysectomized Animals

	Hypophysectomized	Hypophysectomized Growth Hormone	Average Percent- age Gain in Weight
No. of Animals	14	16	
Average Weight in Gm.			
1. Whole Body	173	220	27.2
2. Testis	0.431	0.608	41.1
3. Adrenals	0.0125	0.0161	28.8
4. Ventral Prostate	0.0166	0.0233	40.4
5. Dorsolateral Prostate	0.0279	0.0319	14.3



hypophysectomized animals lose more water and protein and much less fat than normal rats (172). Such a loss in water and protein and consequent loss in tissue weight might not be accompanied by a loss of zinc. This could explain why the zinc concentration, which is based on the wet weight of the tissue, was increased in the cells, liver, adrenals and ventral prostate of the hypophysectomized animals. It would not, however, explain the decreased zinc concentration in the testis and dorsolateral prostate, which would therefore appear to be due to a true reduction in the zinc concentration of these two tissues.

Although the administration of growth hormone to hypophysectomized animals caused no change in the zinc concentration in the testis, ventral prostate and dorsolateral prostate, the administration of growth hormone did cause a marked increase in the weight of these three tissues as shown by Table XXI. If this increase in weight was due only to an increased incorporation of water and protein, the result would amount to a "watering down" of the zinc present and therefore a decrease in zinc concentration. As such was not the case, this suggests that in regard to zinc, growth hormone plays a maintenance role in these tissues. The adrenals from hypophysectomized animals that had received growth hormone showed a significant increase in zinc concentration and a 28.8 per cent gain in weight when compared to hypophysectomized animals. It would appear that the administration of growth hormone to hypophysectomized animals produced a true increase in the zinc concentration in the adrenals. Liver from hypophysectomized animals that had received growth hormone showed a significant decrease in zinc concentration when compared to hypophysectomized animals. This treatment caused the zinc



concentration in the liver to return to normal. It has been reported that a similar result was obtained when ACTH was administered to hypophysectomized animals (8).

The administration of growth hormone to intact animals produced a significant increase in the zinc concentration in the serum and dorsolateral prostate. This was not true of any of the other tissues.

Hypophysectomy caused a significant drop in the corrected specific activity in the serum suggesting an increased withdrawal or loss of the radioactive isotope from the serum. The administration of growth hormone had no effect on this result.

Hypophysectomy produced a significant increase in the relative specific activity in the cells. As hypophysectomy also produced an increased zinc concentration in the cells, and as the specific activity in the cells of hypophysectomized animals was greater than the specific activity in the cells of intact animals, this would suggest that hypophysectomy produces an increased  $\text{Zn}^{65}$  incorporation in the cells.

The result of hypophysectomy on the  $\text{Zn}^{65}$  incorporation in the liver was the same as that shown for cells. There is an increased incorporation of  $\text{Zn}^{65}$  in liver from hypophysectomized animals as compared to intact animals. The administration of growth hormone to hypophysectomized animals produced a significant decrease in both the zinc concentration and relative specific activity in the liver, suggesting a decreased  $\text{Zn}^{65}$  incorporation. The increased relative specific activity in liver from intact animals that received growth hormone is thought to be due to the observed increased zinc concentration in the serum, rather than an increased  $\text{Zn}^{65}$  incorporation.





Hypophysectomy produced a significant increase in the relative specific activity in the testis. Although the specific activity in the testis of hypophysectomized animals was greater than the specific activity in the testis of intact animals, this is thought to be due to the significant decrease in the zinc concentration in the testis of hypophysectomized animals. As a consequence, the increase in the relative specific activity in the testis of hypophysectomized animals would appear to be a result of a change in zinc concentration rather than an increased  $\text{Zn}^{65}$  incorporation.

A decreased relative specific activity was observed in the adrenals of hypophysectomized animals that received growth hormone. As the specific activity in the adrenals from this group of animals is lower than any of the other groups, it would appear that this reduction is due to a decreased  $\text{Zn}^{65}$  incorporation.

Hypophysectomy caused a significant decrease in the relative specific activity in the ventral prostate. As this change is accompanied by a marked reduction in the specific activity in the tissue, it would appear that this is a true decrease in  $\text{Zn}^{65}$  incorporation.

No changes were observed in the relative specific activity in the dorsolateral prostate.



## TISSUE FRACTIONATION STUDIES

Further studies on the zinc present in the tissues studied above led to an investigation of methods for the fractionation of the tissues to establish, if possible, the form in which the zinc is present. Other workers have reported that zinc is tied up with carbonic anhydrase (50, 120, 121, 122), certain other metalloenzymes (111, 114, 115, 116, 117, 119) and with some unidentified protein fractions (3). However, it appeared that the quantities of zinc which can thus be explained, particularly in the dorsolateral prostate, do not account for the amounts of zinc found to be present (173).

A number of methods were used in these studies. Tissues were obtained from intact animals (included in the hormone study) which had been administered  $\text{Zn}^{65}$ . The animals, tissues,  $\text{Zn}^{65}$  and associated methods were those reported under Section III (Methods).

### A. TRICHLOROACETIC ACID METHOD

Twenty-four hours after receiving an intravenous injection of 70 microcuries of  $\text{Zn}^{65}$  the animals were sacrificed and the tissues removed. Tissues that were not used immediately were frozen in an ice chamber.

An accurately weighed amount of tissue was homogenized



in ice-cold 10 per cent zinc-free trichloroacetic acid (TCA) using a glass homogenizer of the Potter-Elvehjem type. The homogenizer tube was placed in an ice bath to maintain a low temperature. Following homogenization the contents of the tube were transferred to a centrifuge tube. After repeated washing of the precipitate an aliquot of the supernatant was taken for zinc analysis and counting purposes (inorganic acid-soluble fraction). Another aliquot of the supernatant was wet ashed using 60 per cent  $\text{HClO}_4$  (total acid-soluble fraction) and zinc concentration and radioactivity determined. Values for the organic acid-soluble fraction were arrived at by subtraction of the values obtained for the inorganic acid-soluble fraction from the values obtained for the total acid-soluble fraction.

After the TCA extraction, the precipitate which remained was washed with cold ethanol, boiled repeatedly with 3:1 ethanol-ether mixture and further washed with ether. An aliquot of the ethanol-ether supernatant was reduced in volume by heating on a water bath and then wet ashed using 60 per cent  $\text{HClO}_4$ . This method is an acceptable procedure for obtaining a separation of lipid-containing material (174). The zinc concentration and radioactivity of this fraction was determined.

The amounts of zinc found in the total acid-soluble fraction, lipid fraction, and residue when added together, resulted in a value much larger than the known zinc concentration of the whole tissue. When the tissues were dry ashed, the results were somewhat better but still not acceptable. It was found that the sensitivity of the dithizone method was not sufficiently great to allow satisfactory measurement of the minute quantities available by these methods.

Nearly all of the radioactivity was found in the acid-soluble





fraction. No difference in radioactivity was observed between the total acid-soluble fraction and the inorganic acid-soluble fraction and no activity was found in the lipid fraction. Only slight activity that might have been due to contamination was found in the residue.

It would appear that the zinc is all in an acid-soluble form.

## B. PAPER STRIP ELECTROPHORESIS METHOD

One hundred microliters of serum was spotted out on paper strips and electrophoresis carried out according to a standard method for the electrophoretic separation of serum proteins (175). The amount of serum applied to the paper was the maximum amount that could be used for suitable resolution with the apparatus available. Following the developing and drying of the strips an attempt was made to locate radioactive areas on the paper. A G-M end-window counter (window thickness 1.4 mg. per cm.<sup>2</sup>) was used to measure activity. It was found initially that there was activity in the sample which was placed on the paper but no significant counts were obtained in any of the spots after separation. Due to the dilution of the serum by the electrolyte and to the separation of the serum into serum protein fractions the activity on the paper strips was diluted beyond the sensitivity of the detecting instrument.

## C. SALTING OUT METHOD

Immediately following the removal of the tissues from the animal, the tissues were homogenized in a Potter-Elvehjem homogenizer with one per cent sodium chloride solution maintained at a





temperature of 0° C. The homogenate was centrifuged for 30 minutes at 3000 r.p.m. and at a constant temperature of 0° C.

A solution of ammonium sulphate saturated at 0° C. was added to aliquots of the supernatant to obtain several degrees of saturation. After the addition of the measured amount of ammonium sulphate, the solutions were allowed to stand for two hours at 0° C. The solutions were then centrifuged, the supernatant decanted and brought to the next desired degree of ammonium sulphate concentration.

It was found that saturation to 44.5 per cent with regard to ammonium sulphate resulted in a heavy precipitate. A further precipitate was obtained on saturation to 64 per cent.

Radioactivity was observed in both precipitates, but continued washing with the same concentrations of ammonium sulphate resulted in complete removal of radioactivity from the precipitates. This would appear to suggest that the zinc associated with these precipitates produced from the saline soluble portion of the tissues was not firmly bound zinc.

#### D. CONTINUOUS FLOW PAPER ELECTROPHORESIS METHOD

Tissues were homogenized with one per cent sodium chloride solution at 0° C. The homogenate was applied at a constant rate to the paper curtain of a continuous flow electrophoresis cell (Model CP, Beckman Instrument, Inc.) which was connected to a constant current supply (Spinco Con-stat). The electrophoresis cell was placed in a cold chamber maintained at 4° C. Background electrolytes employed were five per cent acetic acid and a barbitone



buffer (176). The electrophoresis cell was run continually for 24 hours. Radioactive counting of the collecting tubes was done at two-hour intervals for the first eight hours and a final count was taken at the end of the 24 hour period. The result of plotting counts per minute against tube number was a Gaussian distribution with no indication of any separation into component peaks. The tubes that had a radioactive count also gave a positive reaction with triketohydrindene hydrate (ninhydrin). The solutions from tubes containing radioactivity were pooled, placed in dialysing tubing (Visking) and evaporated to a concentrated solution in a fume hood. This concentrated solution was rerun through the electrophoresis cell. The results were exactly the same as before with no indication of any separation, although both the current and rate of electrolyte flow were varied. Spraying of the paper curtain following electrophoresis with ninhydrin solution showed no indication of separation of amino acid fractions.

A typical result is illustrated in Figure 3 where a liver homogenate was fed to the electrophoresis cell for 24 hours. The background electrolyte was a barbitone buffer of pH 8.6, and the current applied to the paper 25 milliamperes (370-430 volts).

#### E. CHROMATOGRAPHIC AND RADIOAUTOGRAPHIC METHOD

For the chromatographic studies rats were given 140 microcuries of  $\text{Zn}^{65}$  twenty-four hours before killing. The tissues were homogenized and spotted out on chromatographic paper. Both one and two dimensional ascending chromatography was carried out using



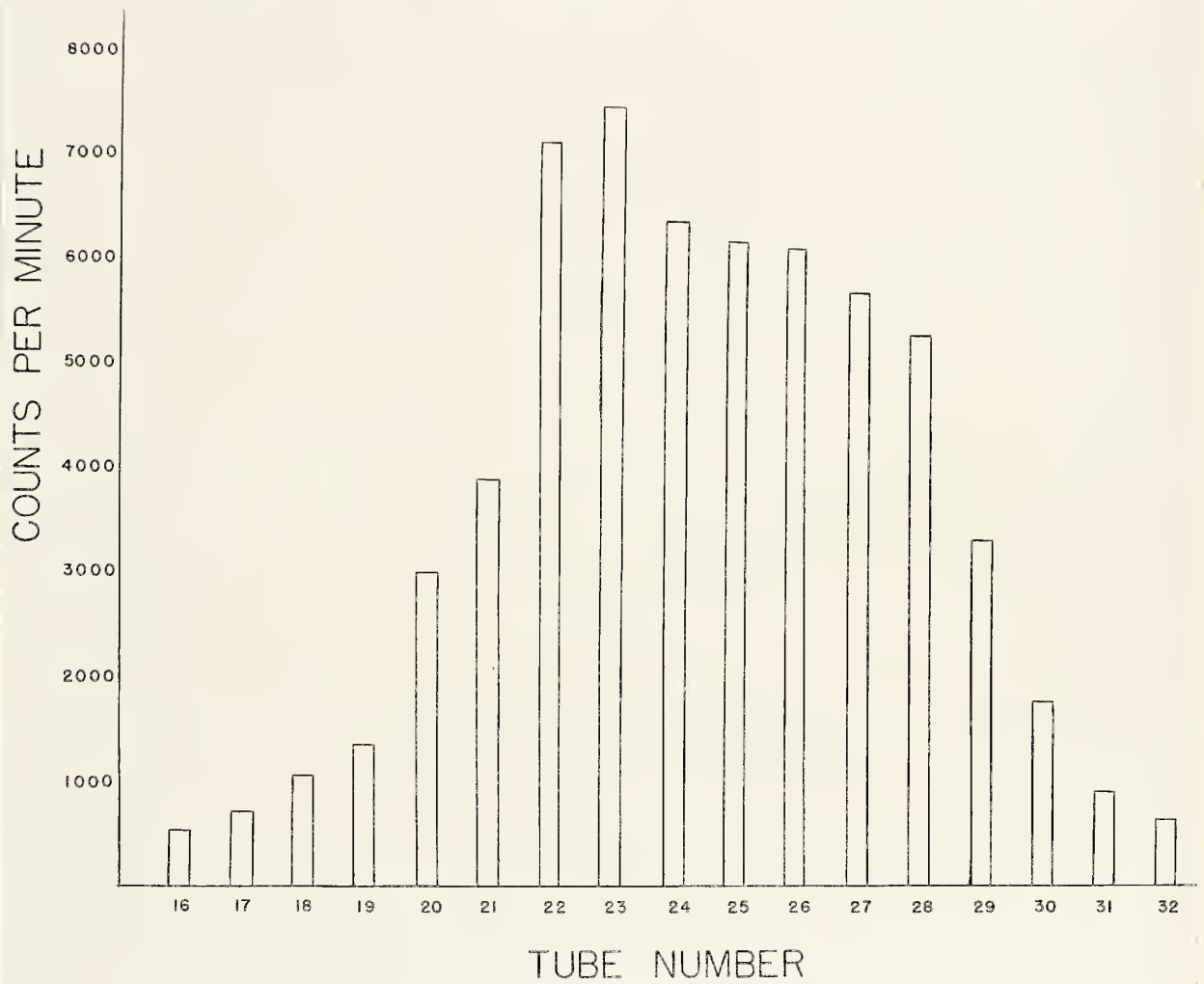


Figure 3.

Counts per minute

versus

tube number of electrophoresis cell.



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a number of different solvents. The papers were sprayed with two per cent ninhydrin in n-butanol. The elution of spots from the chromatograms was accomplished using the elution device of Kamen (177) with deionized water and dilute acetic acid as the eluting solvents. The eluted material was then counted in the scintillation counter. No radioactivity could be detected in any of the ninhydrin spots. As well, the chromatograms were checked directly for radioactivity by placing a G-M end-window tube directly over areas of the chromatogram. This method gave no indication of any radioactive areas present on the chromatograms. Radioautograms of the chromatograms were prepared and resulted in the location of radioactive spots on the papers. The dorsolateral prostate, because of its high zinc content, was selected as the tissue to study in more detail. The fractionation procedure follows.

One part by weight of the dorsolateral prostate was homogenized in five parts by volume of one per cent ice-cold sodium chloride solution. The homogenate was spotted out on 10 x 15 inch Whatman No. 1 filter paper ("For Chromatography") and the spots dried in a current of hot air. The paper was suspended in a glass chromatography chamber for ascending chromatography. The developing solvent was n-butanol 4, glacial acetic acid 1, and deionized water 5. The three liquids were shaken together and the aqueous layer drawn off, placed in a beaker, and the beaker placed in the chamber. The organic layer was poured in the bottom of the chamber and the lower edge of the paper was allowed to dip into the solvent to a depth of 0.25 inches. The direction of the solvent front

a number of different solvents. The papers were sprayed with two per cent ninhydrin in n-butanol. The elution of spots from the chromatograms was accomplished using the elution device of Kamen (177) with

deionized water and dilute acetic acid as the eluting solvents. The eluted material was then counted in the scintillation counter. No radioactivity could be detected in any of the ninhydrin spots. As well, the chromatograms were checked directly for radio

was at 90 degrees to the direction of machining of the paper. Fresh solvent was made every three days because of the problem of esterification of the n-butanol. The chromatogram was developed for 17 hours at room temperature. While still wet the paper was scanned with ultraviolet light (2539A<sup>0</sup>) for the presence of fluorescent spots. The paper was dried, sprayed with a two per cent solution of ninhydrin in n-butanol, and placed in a current of hot air.

A second group of chromatograms were prepared by spotting out the homogenate which had been dialysed for 24 hours against running water (Visking dialysing tube).

Next, the dialysed homogenate was hydrolysed by heating a volume of the homogenate containing approximately 150 milligrams of tissue with 1.5 milliliters of 6N HCl in a sealed glass ampul at a temperature of 110° C. for 24 hours (178). The hydrolysate was dried by heating on a water bath at a reduced pressure, and neutralized if necessary with NaOH solution. The hydrolysate was taken up in a volume of 10 per cent iso-propyl alcohol equal to the volume of homogenate hydrolysed. This solution was spotted out and chromatograms developed.

The hydrolysate solution was dialysed for 24 hours against running water and this dialysed hydrolysate spotted out and chromatograms developed.

A solution of Zn<sup>65</sup> was spotted out on each chromatogram. The solution was diluted by the addition of zinc-free water to contain approximately the same number of counts per minute per unit volume as the substance spotted out.



Radioautograms were prepared by stapling the chromatograms to sheets of Kodak No-Screen Medical X-Ray film. The holes made by the staples served as positioning indicators. The radioautograms were kept between ceramic tiles in complete darkness for a period of 21 days. The films were developed in a tray of Kodak D-11 Developer for five minutes, stop-bath of acetic acid for 30 seconds, and in Kodak Acid Fixing Solution for five minutes. The films were placed in running water for 30 minutes and then dried. Kodak D-19 Developer was also used but did not appear to be superior to Kodak D-11.

Figure 4 shows the chromatogram and corresponding radioautogram of the dorsolateral prostate homogenate. From the radioautogram it is apparent that there are three definite radioactive spots with Rf values different from that of ionic zinc (spot produced by  $\text{Zn}^{65}$  solution). These three radioactive spots correspond to three ninhydrin spots on the chromatogram. There is one faint radioactive spot with the same Rf value as ionic zinc.

On dialysis of the homogenate (Figure 5) no ninhydrin spots are in evidence on the chromatogram indicating complete removal of free amino acids by dialysis. Only one radioactive spot is shown on the radioautogram and this had the same Rf value as the radioactive ionic zinc. As it was found that radioactive ionic zinc can be completely removed from solution by dialysis, this suggests that the radioactive spot is not due to ionic zinc but rather to zinc bound to a non-dialysable protein or polypeptide. The three radioactive spots on the radioautogram of Figure 4 were no longer





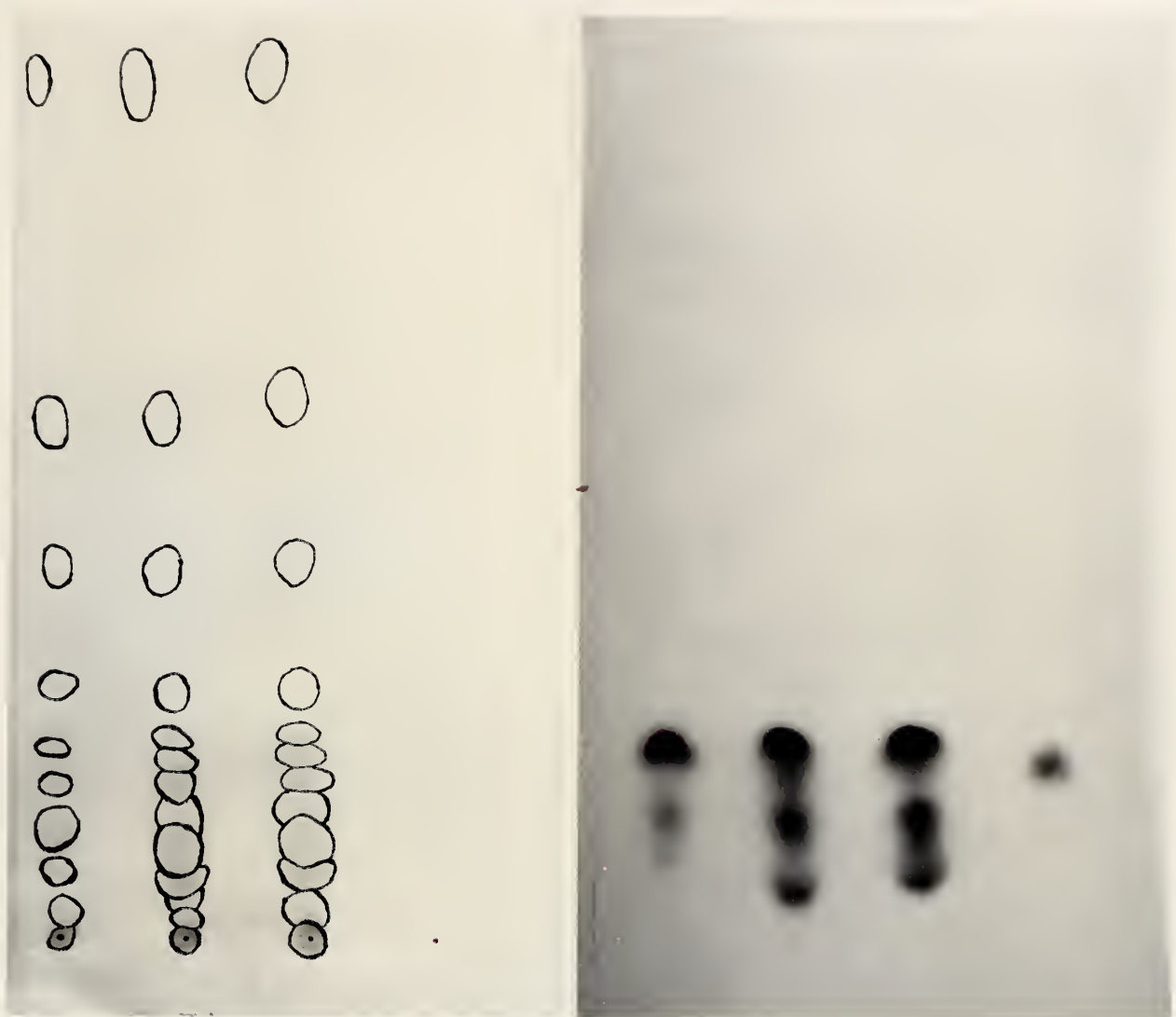


Figure 4.

Chromatogram and radioautogram  
of a sodium chloride suspension of  
the dorsolateral prostate.  
The amount spotted out, from left to right,  
two, three, and one drop of suspension,  
and two drops of  $\text{Zn}^{65}$  solution.





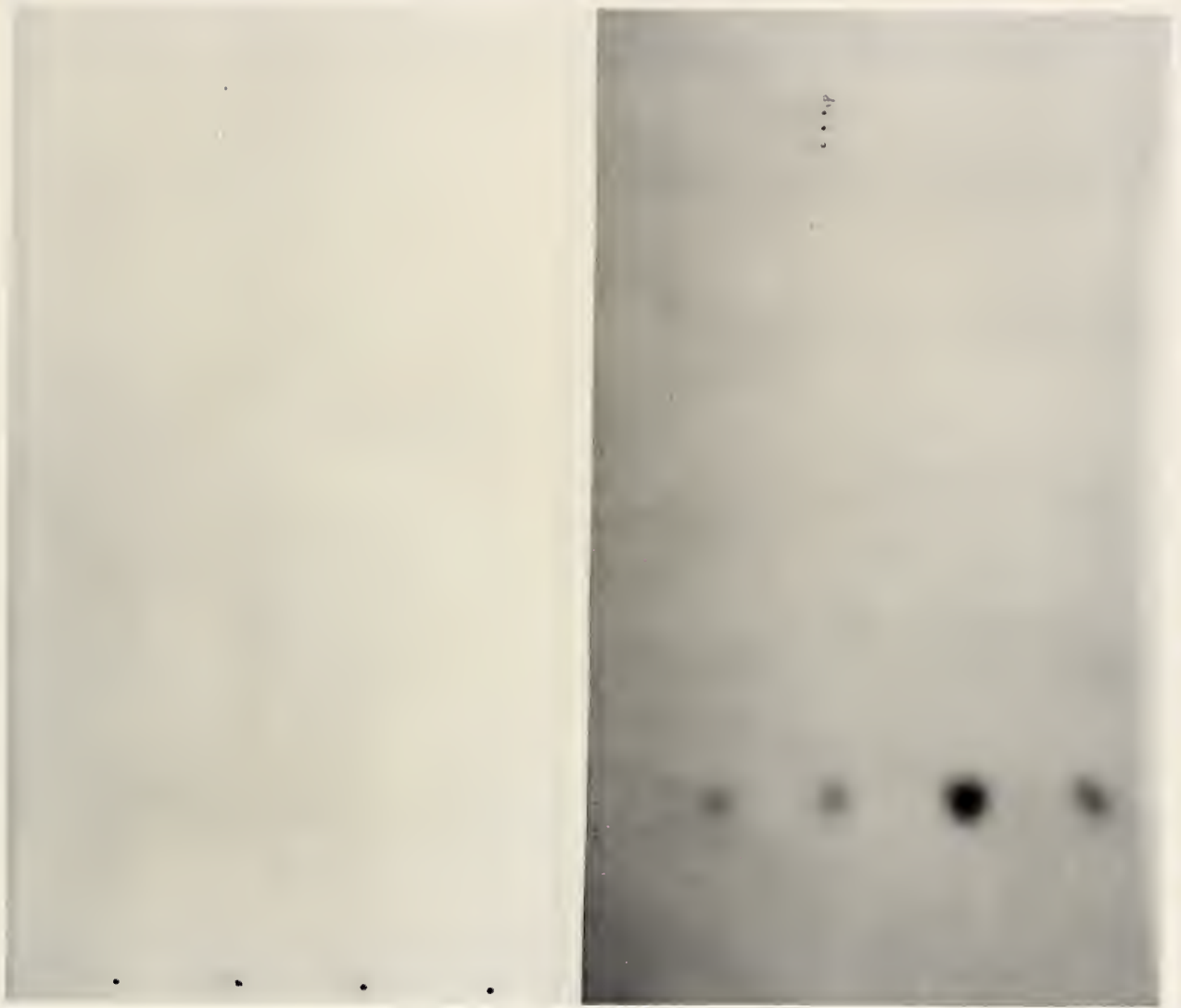


Figure 5.

Chromatogram and radioautogram  
of a dialysed suspension of the dorsolateral prostate.  
The amount spotted out, from left to right, one drop  
of  $\text{Zn}^{65}$  solution, two, four, and  
two drops of dialysed suspension.



present following dialysis which suggests that these radioactive spots were produced by an association of  $\text{Zn}^{65}$  with free amino acids.

Hydrolysis of the dialysed homogenate (Figure 6) resulted in a number of ninhydrin spots on the chromatogram, but only one radioactive spot on the radioautogram. The radioactive spot, which has a markedly different  $R_f$  value from that of ionic zinc, corresponds to a yellow ninhydrin spot on the chromatogram. Two amino acids, proline and hydroxyproline, are known to give a yellow stain with ninhydrin (178). Heavy-metal ions react readily with amino acids and there is evidence that the resulting product may be either a metal salt or a complex involving the amino group (179).

Dialysis of the hydrolysate, as would be expected, resulted in no ninhydrin spots on a chromatogram and no radioactivity on a radioautogram.

Figure 7 is a composite chromatogram and radioautogram.



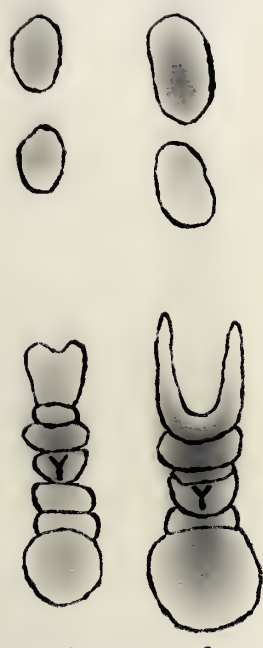


Figure 6.

Chromatogram and radioautogram of a dialysed and hydrolysed suspension of the dorsolateral prostate. The amount spotted out, from left to right, three drops of  $\text{Zn}^{65}$  solution, three, and four drops of dialysed and hydrolysed suspension, and two drops of  $\text{Zn}^{65}$  solution.







Figure 7.

Composite chromatogram and radioautogram. From left to right, one drop of  $\text{Zn}^{65}$  solution, two drops of a sodium chloride suspension of dorsolateral prostate, four drops of dialysed suspension of dorsolateral prostate, four drops of dialysed and hydrolysed suspension of dorsolateral prostate, two and three drops of  $\text{Zn}^{65}$  solution.



## V. GENERAL DISCUSSION

Discussion on individual series is presented at the end of each section. This general discussion is intended to draw these individual discussions together and compare the results with those previously reported by other workers, particularly the work of Rudzik and Riedel (7, 8).

The results obtained with the dorsolateral prostate are of particular interest. A graphic presentation of the state of information now available is presented (Table XXII). Gunn and Gould (5) have suggested that the degree of uptake of  $\text{Zn}^{65}$  by the dorsolateral prostate is an indication of the functional state of the tissue. Rudzik and Riedel (7) have reported that the functional capacity of the dorsolateral prostate in the rat is severely affected by adrenalectomy. They reported a decreased  $\text{Zn}^{65}$  incorporation in adrenalectomized animals. Using the same method for expressing  $\text{Zn}^{65}$  incorporation, the DCA series reported here confirms this observation. Although a different method was used for expressing  $\text{Zn}^{65}$  incorporation in the ACTH study, the conclusion that adrenalectomy causes a decreased  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate, could be drawn. Rudzik and Riedel (7) have reported that the effect of adrenalectomy on  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate could be overcome or corrected by the daily administration of cortisone. This would



TABLE XXII

The Effect of Removal of Certain Endocrine Glands, and the  
Administration of Hormones, on the Zn<sup>65</sup> Incorporation in  
the Dorsolateral Prostate of the Rat

Reported by	Removal of	Result of Removal on Zn <sup>65</sup> Incorporation	Hormone used for Replacement Therapy	Result of Replacement Therapy on Zn <sup>65</sup> Incorporation
Rudzik and Riedel (7)	Adrenals	decrease	cortisone	return to normal
This study	"	decrease	DCA	no apparent effect
This study	"	decrease	ACTH	no apparent effect
Gunn and Gould (6)	Pituitary	decrease	chorionic gonadotropin or testosterone	return to normal
Rudzik and Riedel (8)	"	decrease	ACTH	return to normal
This study	"	no change	growth hormone	no apparent effect
Gunn and Gould (5)	Testes	decrease	testosterone	return to normal



appear to be more or less specific for cortisone because the results obtained with DCA do not show a similar correction of the effect of adrenalectomy. Similarly, it is apparent that in the absence of the adrenals, ACTH does not counteract the effect of adrenalectomy on the zinc in this tissue.

Gunn and Gould (6) have reported that  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate of the rat is decreased following hypophysectomy. Rudzik and Riedel (8) have confirmed this finding. From the results of the growth hormone series reported in this work, it cannot be concluded that hypophysectomy produces a decreased  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate.

The results reported by these three groups of workers are difficult to compare because each is calculated on a different basis. Gunn and Gould (5) report their results in terms of the wet weight of the tissue. Rudzik and Riedel (7, 8) used whole blood as a basis for the calculation of a relative specific activity. In the ACTH and growth hormone series presented in this study, serum  $\text{Zn}^{65}$  was used to obtain a relative specific activity. It may, therefore, not be reasonable to attempt to compare these series in this way.

It does appear, however, that there is a true reduction in the zinc concentration of the dorsolateral prostate of hypophysectomized animals. The administration of chorionic gonadotropin or testosterone has been shown to overcome the effect of hypophysectomy on the  $\text{Zn}^{65}$  incorporation in the dorsolateral prostate (6). Rudzik and Riedel (8) have reported that ACTH has a similar action. From the results obtained by administering growth hormone to hypophysectomized animals, it



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appears that this hormone is, at least in part, necessary for the maintenance of the zinc concentration in the dorsolateral prostate.

Gunn and Gould (5) have reported that in castrated rats,  $Zn^{65}$  incorporation in the dorsolateral prostate is decreased, and that this effect can be prevented by the administration of testosterone.

Testosterone and cortisone, but not DCA, appear to have a direct action on  $Zn^{65}$  incorporation in the dorsolateral prostate. ACTH, in the absence of the adrenal glands, did not increase the  $Zn^{65}$  incorporation in the dorsolateral prostate. ACTH, on the other hand, was shown by Rudzik and Riedel (8) to result in an increase in the  $Zn^{65}$  incorporation in this tissue in hypophysectomized animals, where, of course, the adrenals were present. It would appear that the action of ACTH on  $Zn^{65}$  incorporation in the dorsolateral prostate is due to an action which is mediated by the adrenal gland and not due to a direct action on the dorsolateral prostate.

It is evident, therefore, that zinc metabolism in this tissue is controlled, to a large degree, by hormones of the pituitary and the adrenal gland.

Zinc metabolism of the adrenal gland has been extensively studied in the series reported here, and also by Rudzik and Riedel (7). They reported a marked increase in the zinc concentration in the adrenal gland of intact animals treated daily with cortisone. When the adrenal was studied after the administration of DCA, ACTH, or Growth Hormone, under the conditions reported in this work, no increase in zinc concentration was observed. As was found by the previous workers, intact animals which had been treated with ACTH over a period of 14 days showed an



increased  $\text{Zn}^{65}$  incorporation in the adrenal gland. The suggestion made by Rudzik and Riedel (8) that there is a possible relationship between zinc and adrenal corticosteroid production would appear to be valid. The increased zinc concentration and the decreased  $\text{Zn}^{65}$  incorporation in the adrenals of hypophysectomized animals treated with growth hormone would suggest that growth hormone plays a similar role in this gland to that observed in the dorsolateral prostate. This would seem to be a maintenance role in respect to the supply of zinc in the adrenal gland.

The results of the action of growth hormone on the adrenals of hypophysectomized animals is directly opposite to those obtained when the adrenals are stimulated by ACTH to produce and secrete corticosteroids, as reported by Rudzik and Riedel (8).

From the results of this work and the results obtained by Rudzik (180), it would appear that the administration of cortisone, DCA, ACTH and growth hormone, to intact animals, resulted in no changes in the zinc concentration in the liver. Neither does adrenalectomy nor hypophysectomy produce any true change in the zinc concentration in the liver. However, the administration of ACTH to adrenalectomized animals was shown to produce an increased zinc concentration in the liver, suggesting a direct stimulatory action of ACTH on zinc metabolism in this tissue. This effect was not produced when DCA or cortisone were administered.

The testis appeared to be very resistant to any change in zinc metabolism in the experiments reported. With the exception of the effect of hypophysectomy, which produced a decreased zinc concentration and an increased  $\text{Zn}^{65}$  incorporation, no other changes were observed in the zinc metabolism in the testis. Growth hormone, however, does appear to have





some effect on maintaining the level of zinc in this tissue.

The daily administration of cortisone to intact animals, has been reported by Rudzik and Riedel (7) to produce an increased  $\text{Zn}^{65}$  incorporation in the ventral prostate. A similar effect was observed in this study when ACTH was administered under the same conditions. It would appear that either cortisone or ACTH, but not DCA or growth hormone, produces an increased  $\text{Zn}^{65}$  incorporation in the ventral prostate of intact animals. From the results of this study and those of Rudzik and Riedel (7, 8), neither adrenalectomy nor hypophysectomy appear to have any effect on the zinc concentration in the ventral prostate. However, in agreement with Rudzik and Riedel (8) hypophysectomy does appear to decrease the  $\text{Zn}^{65}$  incorporation in this tissue. As has already been noted in the discussion of the results of the growth hormone series, growth hormone does appear to aid in the maintenance of zinc in the ventral prostate.

The zinc concentration in whole blood was found to be decreased following the daily administration of DCA to intact animals. Rudzik and Riedel (8) reported a similar result when ACTH was administered to intact animals, but not when cortisone was the administered hormone. Although Rudzik and Riedel (7) reported a decreased zinc concentration in whole blood of adrenalectomized animals, this result was not confirmed by this study. The results which they obtained when cortisone was administered to adrenalectomized animals (increased zinc concentration and  $\text{Zn}^{65}$  incorporation in whole blood) are opposite to those which were obtained when DCA was administered, suggesting that the action of DCA and cortisone on zinc metabolism in whole blood is very different.





Intact animals that received growth hormone showed an increased zinc concentration in the serum but no change in the  $\text{Zn}^{65}$  incorporation. It appears that growth hormone produces a build-up of zinc in the serum. No changes were observed in the zinc metabolism in the serum when ACTH was administered to intact animals.

From the results obtained in the ACTH series, it was found that adrenalectomy caused a marked decrease in the zinc concentration and  $\text{Zn}^{65}$  incorporation in the serum. This observation had not been reported previously due to the fact that in the DCA series, and the cortisone study of Rudzik and Riedel (7), whole blood was not separated into serum and cells. The administration of ACTH brought about a return to normal in the zinc concentration and significantly increased the  $\text{Zn}^{65}$  incorporation, which suggests that ACTH has some control at least over the zinc level in the serum. Hypophysectomy did not cause a decrease in the zinc concentration in the serum, although it did produce a decreased  $\text{Zn}^{65}$  incorporation.

The only change observed in the zinc concentration of the cells was an increased concentration in cells from hypophysectomized animals. As was discussed under the growth hormone series, this would not appear to be a true increase, but rather the result of the state of atrophy produced by hypophysectomy. Hypophysectomy and adrenalectomy did produce an increased  $\text{Zn}^{65}$  incorporation in the cells. Intact animals that received a daily injection of ACTH showed an increased  $\text{Zn}^{65}$  incorporation in the cells, but in adrenalectomized animals the administration of ACTH had the opposite effect. It would appear that the action of ACTH in intact animals on  $\text{Zn}^{65}$  incorporation in the cells, is an indirect action mediated through the adrenals.

From the fractionation studies, it would appear that a large amount



of the zinc present in the dorsolateral prostate is in a readily-dialysable form. The remainder of the zinc appears to be associated with a non-dialysable polypeptide or protein, the hydrolysis of which produces a zinc-containing amino acid.



## VI CONCLUSIONS

1. The action of desoxycorticosterone acetate on zinc metabolism in the tissues studied; dorsolateral prostate, liver, adrenals, testis, ventral prostate and the blood; is shown to be markedly different from that of cortisone as previously reported (7).
2. The effect of growth hormone when administered to hypophysectomized animals was to maintain the zinc concentration in the testis, ventral and dorsolateral prostate, and to produce a significant increase in the zinc concentration in the adrenals. It is suggested that growth hormone plays an important role in the maintenance of zinc balance in these tissues.
3. The results obtained with adrenocorticotrophic hormone on  $Zn^{65}$  incorporation in the dorsolateral prostate suggest that the action of adrenocorticotrophic hormone is mediated by the adrenal gland and is not due to a direct action on the tissue.
4. The results obtained from this study on the metabolism of zinc in the adrenal glands further substantiates the proposal previously advanced by Rudzik and Riedel (8) that there is a possible relationship between zinc and adrenal corticosteroid formation.
5. The administration of adrenocorticotrophic hormone to adrenalectomized animals resulted in a marked increase in the zinc concentration in the liver suggesting a stimulatory action on zinc metabolism.





in the liver by this hormone.

6. The daily administration of desoxycorticosterone acetate to intact animals produced a decreased zinc concentration in whole blood. The administration of desoxycorticosterone acetate to adrenalectomized animals produced a decreased zinc concentration and  $\text{Zn}^{65}$  incorporation in whole blood.
7. The administration of growth hormone to intact animals caused an increased zinc concentration in the serum with no effect on the  $\text{Zn}^{65}$  incorporation. This suggests a build-up of zinc in the serum brought about by growth hormone.
8. Adrenalectomy produced a decreased zinc concentration and  $\text{Zn}^{65}$  incorporation in the serum. As adrenocorticotrophic hormone corrected this effect it appears that this hormone exercises some control over the zinc level in this tissue.
9. Hypophysectomy and adrenalectomy produced an increased  $\text{Zn}^{65}$  incorporation in the blood cells. The daily administration of adrenocorticotrophic hormone to intact animals produced an increased  $\text{Zn}^{65}$  incorporation in the cells. The opposite effect was produced when adrenocorticotrophic hormone was administered to adrenalectomized animals. It appears that the action of this hormone on  $\text{Zn}^{65}$  incorporation in the cells of intact animals is a result of an indirect action mediated through the adrenals.
10. A large portion of the zinc present in the dorsolateral prostate is in a readily-dialysable form and may be associated by complex formation with free amino acids. The remainder of the zinc appears to be associated with a polypeptide or protein which on hydrolysis yields a zinc-containing amino acid fraction.





BIBLIOGRAPHY

1. "Documenta Geigy Scientific Tables", 5th Ed. Jesse Broad and Co. Ltd. (England) 1956, p. 87.
2. Keilin, D., and Mann, T., Nature 144, 442, 1939.
3. Vallee, B. L., Physiol. Rev., 39, 443, 1959.
4. Mawson, C. A. and Fischer, M. I., Nature 167, 859, 1951.
5. Gunn, S. A., and Gould, T. C., Endocrinology 58, 443, 1956.
6. Gunn, S. A., and Gould, T. C., J. Endocrin. 16, 18, 1957.
7. Rudzik, A. D., and Riedel, B. E., Can. J. Biochem. Physiol. 38, 845, 1960.
8. Rudzik, A. D., and Riedel, B. E., Can. J. Biochem. Physiol. 38, 1003, 1960.
9. Raulin, J., Ann. sci. nat. Botan. et biol. vegetale 11, 93, 1869; through Lutz, R. E., J. Ind. Hyg. 8, 177, 1926.
10. Lechartier, G., and Bellamy, F., Compt. rend. Acad. Sci. 84, 687, 1877; through Underwood, E. J., Trace Elements in Human and Animal Nutrition, New York, Acad. Press, 1956.
11. Raoult, F., and Breton, H., Compt. rend. Acad. Sci. 85, 40, 1877; through Vallee, B. L., Physiol. Rev. 39, 443, 1959.
12. Bradley, H. C., Science 19, 196, 1904; through Underwood, E. J., Trace Elements in Human and Animal Nutrition, New York, Acad. Press, 1956.
13. Mendel, L. B., and Bradley, H. C., Am. J. Physiol. 14, 313, 1905; through Underwood, E. J., Trace Elements in Human and



Animal Nutrition, New York, Acad. Press, 1956.

14. Bertrand, G. , and Javillier, M. , Compt. rend. 152, 900, 1911;  
through Underwood, E. J. , Trace Elements in Human and Animal  
Nutrition, New York, Acad. Press, 1956.
15. Lutz, R. E. , J. Ind. Hyg. 8, 177, 1926.
16. Drinker, K. R. , and Collier, E. S. , J. Ind. Hyg. 8, 257, 1926.
17. Bertrand, G. , and Vladesco, R. , Compt. rend. Acad. Sci. 54,  
176, 1921; through Drinker, K. R. , and Collier, E. S. , J. Ind.  
Hyg. 8, 257, 1926.
18. Bertrand, G. , and Benzon, B. , Compt. rend. Acad. Sci. 175,  
289, 1922; through Todd, W. R. , Elvehjem, C. A. , and Hart,  
E. B. , Am. J. Physiol. 107, 146, 1934.
19. McHargue, J. S. , Am. J. Physiol. 77, 245, 1926.
20. Hubbell, R. , and Mendel, L. B. , J. Biol. Chem. 75, 567, 1927.
21. Todd, W. R. , Elvehjem, C. A. , and Hart, E. B. , Am. J. Physiol.  
107, 146, 1934.
22. Stirn, F. E. , Elvehjem, C. A. , and Hart, E. B. , J. Biol. Chem.  
109, 347, 1935.
23. Finch, A. H. , and Kinnison, A. F. , Univ. Arizona Agric. Exp.  
Stat. , Tech. Bull. 47, 1933; through Stirn, F. E. , Elvehjem, C. A. ,  
and Hart, E. B. , J. Biol. Chem. 109, 347, 1935.
24. Barnette, R. M. , and Warner, J. D. , Soil Sci. 39, 145, 1935.
25. Keilin, D. , and Mann, T. , Nature, 153, 107, 1944.
26. Tucker, H. F. , and Salmon, W. D. , Proc. Soc. Exp. Biol. Med.  
88, 613, 1955.



27. Vallee, B. L. , Wacker, W. E. C. , Bartholomay, A. F. , and Robin, E. D. , New Eng. J. Med. 255, 403, 1956.
28. Vallee, B. L. , Wacker, W. E. C. , Bartholomay, A. F. , and Hoch, F. L. , New Eng. J. Med. 257, 1055, 1957.
29. Ballou, J. E. , AEC Research and Development Report, Document HW - 63047, Richland, Washington, 1960 (Unclassified).
30. Feaster, J. P. , Hansard, S. L. , McCall, J. T. , and Davis, G. K. , Am. J. Physiol. 181, 287, 1955.
31. Drinker, K. R. , Fehnel, J. W. , and Marsh, M. , J. Biol. Chem. 72, 375, 1927.
32. McCance, R. A. , and Widdowson, E. M. , Biochem. J. 36, 692, 1942.
33. Millar, M. J. , Fischer, M. I. , Mawson, C. A. , and Elcoate, P. V. , Nature, 174, 881, 1954.
34. Mager, M. , McNary, W. F. , and Lionetti, F. , J. Histochem. and Cytochem. 1, 493, 1953.
35. Montgomery, M. L. , Sheline, G. E. , and Chaikoff, I. L. , J. Exp. Med. 78, 151, 1943.
36. Birnstingl, M. , Stone, B. , and Richards, V. , Am. J. Physiol. 186, 377, 1956.
37. Drinker, K. R. , Thompson, P. K. , and Marsh, M. , Am. J. Physiol. 80, 31, 1927.
38. Scoular, F. I. , J. Nutrition 17, 103, 1939.
39. Stern, A. , Nalder, M. , and Macy, I. G. , J. Nutrition Supp. 21, 8, 1941.





40. Tribble, H. M., and Scoular, F. I., J. Nutrition, 52, 209, 1954.
41. Sheline, G. E., Chaikoff, I. L., Jones, H. B., and Montgomery, M. L., J. Biol. Chem. 147, 409, 1943.
42. Day, H. G., Fed. Proc. 1, 188, 1942.
43. Nishimura, H., J. Nutrition 49, 79, 1953.
44. Imada, S., Jikkenshokibyogaku 15, 157, 1940; through Nishimura, H., J. Nutrition 49, 79, 1953.
45. Hove, E., Elvehjem, C. A., and Hart, E. B., Am. J. Physiol. 119, 768, 1937.
46. Hove, E., Elvehjem, C. A., and Hart, E. B., Am. J. Physiol. 124, 750, 1938.
47. Follis, R. H., Day, H. G., and McCollum, E. V., J. Nutrition 22, 223, 1941.
48. Day, H. G., and McCollum, E. V., Proc. Soc. Exp. Biol. 45, 282, 1940.
49. Hove, E., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem. 134, 425, 1940.
50. Hove, E., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem. 136, 425, 1940.
51. Wachtel, L. W., Hove, E., Elvehjem, C. A., and Hart, E. B., J. Biol. Chem. 138, 361, 1941.
52. Millar, M. J., Fischer, M. I., Elcoate, P. V., and Mawson, C. A., Can. J. Biochem. Physiol. 36, 557, 1958.
53. Parizek, J., Nature 177, 1036, 1956.
54. Parizek, J., J. Endocrin. 15, 56, 1957.
55. Kernkamp, H. C. H., and Ferrin, E. F., J. Am. Vet. Med. Assoc. 123, 217, 1953.



56. Lewis, P. K., Hoekstra, W. G., Grummer, R. H., and Phillips, P. H., J. Animal Sci. 15, 741, 1956.
57. Luecke, R. W., Hoefer, J. A., Brammell, W. S., and Thorp, F., J. Animal Sci. 15, 347, 1956.
58. Lewis, P. K., Hoekstra, W. G., and Grummer, R. H., J. Animal Sci. 16, 578, 1957.
59. Stevenson, J. W., and Earle, I. P., J. Animal Sci. 15, 1036, 1956.
60. Luecke, R. W., Hoefer, J. A., Brammell, W. S., and Schmidt, D. A., J. Animal Sci. 16, 3, 1957.
61. Conrad, J. H., and Beeson, W. M., J. Animal Sci. 16, 589, 1957.
62. Newland, H. W., Ullrey, D. E., Hoefer, J. A., and Luecke, R. W., J. Animal Sci. 17, 886, 1958.
63. Hoekstra, W. G., Lewis, P. K., Phillips, P. H., and Grummer, R. H., J. Animal Sci. 15, 752, 1956.
64. Eggleton, W. G. E., Biochem. J. 33, 403, 1939.
65. Eggleton, W. G. E., Biochem. J. 34, 991, 1940.
66. Smith, S. E., Larson, E. J., J. Biol. Chem. 163, 29, 1946.
67. Sutton, W. R., and Nelson, V. E., Proc. Soc. Exp. Biol. Med. 36, 211, 1937.
68. Duncan, G. D., Gray, L. F., and Daniel, L. J., Proc. Soc. Exp. Biol. Med. 83, 625, 1953.
69. Myers, V. C., Beard, H. H., J. Biol. Chem. 94, 89, 1931.
70. Sadasivan, V., Biochem. J. 48, 527, 1951.
71. Sadasivan, V., Biochem. J. 52, 452, 1952.
72. Van Reen, R., Arch. Biochem. and Biophys. 46, 337, 1953.



73. Gray, L. F., Ellis, G. H., J. Nutrition 40, 441, 1950.
74. Sadasivan, V., Biochem. J. 49, 186, 1951.
75. Scott, D. A., and Fisher, A. M., Am. J. Physiol. 121, 253, 1938.
76. Vallee, B. L., Fluharty, R. G., and Gibson, J. G., Acta contra Cancerum 6, 869, 1949.
77. Itallie, V., Ned. Tijdschr. Geneesk. 63, 1709, 1907; through Scott, D. A., and Fisher, A. M., Am. J. Physiol. 121, 253, 1938.
78. Widdowson, E. M., McCance, R. A., and Spray C. M., Clin. Sci. 10, 113, 1951.
79. Vallee, B. L., Am. Med. Assoc. Arch. Industrial Health 16, 147, 1957.
80. Vallee, B. L., and Altschule, M. D., Physiol. Rev. 29, 370, 1949.
81. Tauber, F. W., and Krause, A. C., Am. J. Ophth. 26, 260, 1943.
82. Bowness, J. M., Morton, R. A., Shakir, M. H., and Stubbs, A. L., Biochem. J. 51, 521, 1952.
83. Bowness, J. M., and Morton, R. A., Biochem. J. 51, 530, 1952.
84. Vallee, B. L., and Gibson, J. G., J. Biol. Chem. 176, 445, 1948.
85. Vallee, B. L., and Altschule, M. D., Blood 4, 398, 1949
86. Vikbladh, I., Scand. J. Clin. Lab. Invest. Suppl. 2, 1951.
87. Hoch, F. L., and Vallee, B. L., J. Biol. Chem. 195, 531, 1952.
88. Tupper, R., Watts, R. W. E., and Wormall, A., Biochem. J. 48, XXXVII, 1951.
89. Mager, M., and Lionetti, F., Fed. Proc. 13, 258, 1954.
90. Koch, H. J., Smith, E. R., and McNeely, J., Cancer 10, 151, 1957.
91. Koch, H. J., Smith, E. R., Shimp, N. F., and Connor, J., Cancer 9, 499, 1956.





92. Vallee, B. L. , and Gibson, J. G. , Blood 4, 455, 1949.
93. Vallee, B. L. , and Fluharty, R. G. , J. Clin. Invest. 26, 1199, 1947.
94. Wolff, H. P. , Klin. Wochschr. 34, 409, 1956; through Chemical Abstracts 50, 17113, 1956.
95. Vikbladh, I. , Scand. J. Clin. Lab. Invest. 2, 143, 1950.
96. Vesell, E. S. , and Bearn, A. G. , Proc. Soc. Exp. Biol. Med. 94, 96, 1957.
97. Surgenor, D. M. , Koechlin, B. A. , and Strong, L. E. , J. Clin. Invest. 28, 73, 1949.
98. Smirnov, A. A. , Biokhimiya 13, 79, 1948; through Chemical Abstracts 42, 8302, 1948.
99. Berfenstam, R. A. , Acta paediat. 87, Suppl. , 1952; through Vallee, B. L. , Physiol. Rev. 39, 443, 1959.
100. Stevenson, S. S. , J. Clin. Inv. 22, 403, 1943.
101. Feaster, J. P. , Hansard, S. L. , McCall, J. T. , Skipper, F. H. , and Davis, G. K. , J. Animal Sci. 13, 781, 1954.
102. Keilin, D. , and Mann, T. , Biochem. J. 34, 1163, 1940.
103. Vallee, B. L. , Lewis, H. D. , Altschule, M. D. , and Gibson, J. G. , Blood 4, 467, 1949.
104. Mawson, C. A. , and Fischer, M. I. , Can. J. Med. Sci. 30, 336, 1952.
105. Mawson, C. A. , and Fischer, M. I. , Biochem. J. 55, 696, 1953.
106. Gunn, S. A. , Gould, T. C. , Ginori, S. S. , and Morse, J. G. , Proc. Soc. Exp. Biol. Med. 88, 556, 1955.





107. Wakeley, J. C. N. , Moffatt, B. , Crook, A. , and Mallard, J. R. ,  
Internat. J. App. Rad. Isotopes 7, 225, 1960.
108. Gunn, S. A. Gould, T. C. , Am. J. Physiol. 193, 505, 1958.
109. Prout, G. R. , Daniel, O. , and Whitmore, W. F. , J. Urol. 78,  
471, 1957.
110. Mawson, C. A. , and Fischer, M. I. , Arch. Biochem. Biophys.  
36, 485, 1952.
111. Vallee, B. L. , and Hoch, F. L. , J. Am. Chem. Soc. 77, 821, 1955.
112. Vallee, B. L. , and Hoch, F. L. , Proc. Nat. Acad. Sci. 41, 327,  
1955.
113. Theorell, H. , Nygaard, A. P. , and Bonnichsen, R. K. , Acta  
Chem. Scand. 9, 1148, 1955.
114. Vallee, B. L. , Hoch, F. L. , Fed. Proc. 15, 619, 1956.
115. Vallee, B. L. , Adelstein, S. J. , and Olson, J. A. , J. Am. Chem.  
Soc. 77, 5196, 1955.
116. Vallee, B. L. , and Wacker, W. E. C. , J. Am. Chem. Soc. 78,  
1771, 1956.
117. Vallee, B. L. , and Neurath, H. , J. Am. Chem. Soc. 76, 5006,  
1954.
118. Vallee, B. L. , and Neurath, H. , J. Biol. Chem. 217, 253, 1955.
119. Mathies, J. C. , J. Biol. Chem. 233, 1121, 1958.
120. Mann, T. , and Keilin, D. , Proc. Roy. Soc. London, Series B,  
126, 303, 1938.
121. Scott, D. A. , and Mendive, J. R. , J. Biol. Chem. 139, 661,  
1941.



122. Scott, D. A. , and Mendive, J. R. , J. Biol. Chem. 140, 445, 1941.
123. Tupper, R. , Watts, R. W. E. , and Wormall, A. , Biochem. J. 50, 429, 1952.
124. Scott, D. A. , and Fisher, A. M. , J. Biol. Chem. 144, 371, 1942.
125. Petermann, M. N. , and Hakala, N. V. , J. Biol. Chem. 145, 701, 1942.
126. Smith, E. C. B. , Biochem. J. 34, 1176, 1940.
127. Meldrum, N. V. , and Roughton, F. J. W. , J. Physiol. 80, 113, 1933.
128. Webb, E. C. , van Heyningen, R. , Biochem. J. 41, 74, 1947.
129. Mann, T. , and Keilin, D. , Nature 146, 164, 1940.
130. Davenport, H. W. , J. Physiol. 97, 32, 1939.
131. Fischer, M. I. , Tikkala, A. O. , and Mawson, C. A. , Can. J. Biochem. Physiol. 33, 181, 1955.
132. Day, R. , and Franklin, J. , Science 104, 363, 1946.
133. Hayes, J. E. , and Velick, S. F. , J. Biol. Chem. 207, 225, 1954.
134. Hoch, F. L. , and Zotos, B. , Fed. Proc. 16, 359, 1957.
135. Hoch, F. L. , and Vallee, B. L. , J. Biol. Chem. 221, 491, 1956.
136. Vallee, B. L. , Kaagi, J. H. R. , and Hoch, F. L. , Fed. Proc. 17, 326, 1958.
137. Theorell, H. , and Bonnichsen, R. , Acta Chem. Scand. 5, 1105, 1951.
138. Ehrenberg, A. , Acta Chem. Scand. 11, 1257, 1957.



139. Vallee, B. L., and Hoch, F. L., J. Biol. Chem. 225, 185, 1957.
140. Olson, J. A., and Anfinsen, C. B., J. Biol. Chem. 197, 67, 1952.
141. Adelstein, S. J., and Vallee, B. L., J. Biol. Chem. 233, 589, 1958.
142. Adelstein, S. J., and Vallee, B. L., J. Biol. Chem. 234, 824, 1959.
143. Ito, K., Osaka Daigaku Igaku Zasshi 9, 1059, 1957; through Chemical Abstracts 52, 4726, 1958.
144. Putnam, F. W., and Neurath, H., J. Biol. Chem. 166, 603, 1946.
145. Vallee, B. L., Rupley, J. A., Coombs, T. L., and Neurath, H., J. Am. Chem. Soc. 80, 4750, 1958.
146. Scott, D. A., Biochem. J. 28, 1592, 1934.
147. Fisher, A. M., and Scott, D. A., Biochem. J. 29, 1055, 1935.
148. Evans, J. S., Hines, L. R., Ceithaml, J. J., and Koch, F. C., Endocrinology 26, 1012, 1940.
149. Bischoff, F., Am. J. Physiol. 121, 765, 1938.
150. Fevold, H. L., Hisaw, F. L., and Greep, R., Am. J. Physiol. 117, 68, 1936.
151. Leathem, J. H., Am. J. Physiol. 145, 28, 1945.
152. Holtermann, H., Heier, A. and Bergh, H., Lancet 262, 1308, 1952.
153. Kocsis, J. J., Walaszek, E. J., Graham, C. E., and Geiling, E. M. K., Fed. Proc. 12, 336, 1953.





154. Carr, J. E., Conn, J. B., and Wartman, T. G., Science 116, 566, 1952.
155. Millar, M. J., Elcoate, P. V., and Mawson, C. A., Can. J. Biochem. Physiol. 35, 865, 1957.
156. Hall, C. E., and Hall, O., Endocrinology 63, 329, 1958.
157. Boces, N. F., Endocrinology 63, 323, 1958.
158. Gorby, C. K., Leonard, C. A., Ambrus, J. L., and Harrison, J. W. E., J. Am. Pharm. Assoc., Sci. Ed. 42, 213, 1953.
159. Riedel, B. E., Logan, J. E., and Rossiter, R. J., Endocrinology 55, 219, 1954.
160. Riedel, B. E., "Effect of Hypophysectomy and the Administration of ACTH on the Phosphorous Metabolism of the Adrenal and the Liver of the Rat" Ph.D. Thesis, University of Western Ontario, London, Canada, 1953.
161. Millar, M. J., Elcoate, P. V., Fischer, M. I., and Mawson, C. A., Can. J. Biochem. Physiol. 38, 1457, 1960.
162. Kagi, J. H. R., and Vallee, B. L., Anal. Chem. 30, 1951, 1958.
163. Pijck, J., Hoste, J., and Gillis, J., Mededel. Koninkl. Vlaam. Acad. Wetenschap., Kl. Wetenschap. 20, #8, 1958; through Chemical Abstracts 53, 11503, 1959.
164. Fairhall, J. T., J. Ind. Hyg. 8, 165, 1926.
165. The Pharmacopeia of the United States of America, Sixteenth Revision, Mack Printing Company, Easton, Pa., 1960, p. 913.
166. Meites, L., "Polarographic Techniques", Interscience, New York, 1955.



167. Ahrens, L. H. , "Spectrochemical Analysis", Addison-Wesley, Cambridge, Mass. , 1950.
168. Banks, T. E. , Tupper, R. , White, E. M. A. , and Wormall, A. , Internat. J. App. Rad. Isotopes 4, 221, 1959.
169. Vallee, B. L. , and Gibson, J. G. , J. Biol. Chem. 176, 435, 1948.
170. Milton, R. F. , and Waters, W. A. , "Methods of Quantitative Micro-Analysis", 2nd Ed. , Edward Arnold Ltd. , London, 1955.
171. Wilks, S. C. , "Elementary Statistical Analysis", Princeton University Press, Princeton, New Jersey, 1952.
172. Lee, M. O. , and Ayres, G. B. , Endocrinology, 20, 489, 1936.
173. Mawson, C. A. , and Fischer, M. I. , Proceed. 11e Congres International de Biochimie, Paris, 21-27 Juillet 1952, p. 145.
174. Schneider, W. C. , J. Biol. Chem. 161, 293, 1945.
175. Manual "Instructions for the Spinco Model "R" Paper Electrophoresis Apparatus", Belmont, California.
176. Manual "Continuous Flow Paper Electrophoresis Cell Model CP", Beckman Instrument, Belmont, California.
177. Kamen, M. D. , "Isotopic Tracers in Biology", 3rd Ed. , Academic Press Inc. Publishers, New York, 1957, p. 412.
178. Cramer, F. , "Paper Chromatography", 2nd Ed. , MacMillan and Co. Ltd. , London, 1954, p. 46.
179. Fox, S. W. , and Foster, J. F. , "Introduction to Protein Chemistry", John Wiley and Sons, Inc. , New York, 1957, p. 56.



180. Rudzik, A. D. , "The Metabolism of Zinc in the Sex Glands and Adrenals of the Male Rat Using  $\text{Zn}^{65}$  as a Tracer", M.Sc. Thesis, University of Alberta, Edmonton, Canada, 1958.



## APPENDIX A





TABLE I

Whole Blood - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				DCA (Chronic)				DCA (Acute)			
	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity
	257	9.4	3493	89.8	225	5.0	2765	62.2	339	9.6	3881	131.6
	328	7.3	4040	132.5	280	5.2	3883	108.7	328	7.0	5554	182.2
	252	9.3	3837	96.7	265	5.9	4156	110.1	288	9.4	3765	108.4
	335	5.6	4433	148.5	267	5.3	3980	106.3	256	6.6	2443	62.5
	330	6.2	3749	123.7	244	4.1	3237	79.0	220	6.1	3333	73.3
	328	5.8	4388	143.9	290	4.5	3157	91.6	217	5.0	3680	79.9
	243	8.5	3170	77.0					195	5.1	3197	62.3
	236	7.8	4384	103.5								
	343	5.7	4144	142.1								
	335	11.9	3854	129.1								
	325	7.8	3565	115.9								
	277	10.1	4321	119.7								
	225	9.8	3955	89.0								
	233	6.3	3303	77.0								
Mean	289	8.0	3903	113.5	262	5.0	3530	93.0	263	7.0	3693	100.0
S. D.		$\pm 1.9$	$\pm 412$	$\pm 25$		$\pm 0.6$	$\pm 553$	$\pm 19$		$\pm 1.9$	$\pm 952$	$\pm 44$



TABLE II

## Whole Blood - Adrenalectomized Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized				DCA (Chronic)				DCA (Acute)			
	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corr. Specific Activity
	267	9.1	4370	116.8	300	7.6	2005	60.2	308	9.1	3148	97.0
	283	9.5	4797	135.8	236	5.6	4485	105.8	364	8.2	2492	90.7
	295	7.6	4061	119.8	255	6.6	4784	122.0	341	6.4	4747	161.9
	286	7.7	3610	103.2	255	5.3	5108	130.3	352	6.1	3790	133.4
	294	6.1	4489	132.0	245	6.4	4081	100.0	363	5.2	5041	183.0
	295	7.0	4481	132.2					328	8.1	4128	135.4
	316	8.3	6150	194.3								
	321	5.3	4362	140.0								
	241	9.2	3633	87.6								
	311	8.0	3607	112.2								
	281	8.9	3051	85.7								
	315	6.7	3316	104.5								
	304	9.2	3289	100.0								
	254	8.8	4121	104.7								
	220	7.8	4104	90.3								
Mean	286	7.9	4096	117.3	258	6.3	4093	103.7	343	7.2	3891	133.6
S. D.		$\pm 1.2$	$\pm 765$	$\pm 28$		$\pm 0.9$	$\pm 1227$	$\pm 27$		$\pm 1.5$	$\pm 962$	$\pm 36$



TABLE III

Liver - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				DCA (Chronic)		DCA (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	46.9 41.0 45.3 69.7 50.9 62.2 42.1 41.0 46.9 42.0 48.3 45.6 72.9 48.3 45.6 43.3 44.3	8093 6829 7491 5017 7589 6638 8666 6935 6524 6750 5646 6192 5618 3508 5583 3149 7402	36.8 48.6 57.9 49.2 41.6 62.0 47.4 36.4 43.9 43.3 51.4 53.0 43.5 41.7 44.3 51.1	6716 7484 6305 6579 9907 10891 11546 5709 5564 6280 6343 5739 4783 7225 6746 8872	46.9 44.9 39.6 43.6 50.9 86.9 54.1 59.0 55.5 50.8	8129 9485 9075 7213 8610 9502 5640 7171 4093 6681	43.8 41.7 47.0 40.4 42.4 45.8 36.1 52.8 48.2 43.9	9242 12663 9850 4915 6174 6255 6966 5907 7250 7531
Mean			47.7	7309	56.3	7559	44.2	7675
S. D.			± 8.8	± 1878	±13.6	± 1762	± 4.9	± 2455





TABLE IV

## Liver -Adrenalectomized Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		DCA (Chronic)		DCA (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	50.9 45.2 51.8 54.2 55.1 43.9 69.4 46.2 40.9	8638 7883 7719 7811 7360 7583 8888 7708 7520	40.7 46.9 43.5 43.9 46.8 38.6 76.6 69.3 64.1 55.7 54.6 51.1	6569 6493 6659 5758 6044 8301 5560 4808 8561 6230 6240 6115	42.0 54.6 46.9 41.5 46.1 53.3 53.2 50.2 47.0 34.3	6421 7341 6340 9518 7990 9568 7382 5815 7292 6611
Mean	50.9	8110	52.6	6444	46.9	7427
S. D.	± 8.4	± 642	±12.4	± 1053	± 6.4	± 1281



TABLE V

Testis - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				DCA (Chronic)		DCA (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	23.1 28.7 24.9 30.8 26.3 25.8 30.6 26.1 27.2 23.6 34.1 27.5 18.9 26.9 27.5 26.1 27.8	2850 2837 2267 2103 2367 2607 2227 2062 2582 2025 2051 1229 1427 1765 1809 2078 2469	33.5 33.0 29.5 35.0 32.1 29.0 30.4 29.6 27.2 29.7 28.1 29.5 32.3 23.3 27.4 26.2	2844 2396 3146 2845 3502 1709 2029 2069 1883 1985 2115 2305 2245 1919 2839 --	25.7 25.9 25.7 26.9 25.9 33.8 30.4 35.4 28.4 30.5 29.4	2687 2590 2250 2244 2168 1578 1091 1189 2038 942 1734	27.1 26.6 26.3 29.8 29.2 27.1 25.8 23.0 30.1 28.8	2257 2157 2117 1953 2442 2456 3064 2556 2448 1726
Mean			29.9	2379	28.9	1864	27.4	2318
S. D.			± 3.8	± 517	± 3.4	± 602	± 2.2	± 368



TABLE VI

Testis - Adrenalectomized Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		DCA (Chronic)		DCA (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	26.7 28.6 26.8 24.9 25.7 27.9 27.1 27.2 28.3	2369 2595 2358 2649 2434 3138 2940 3291 3090	26.2 27.9 27.0 28.5 27.5 25.8 28.8 28.5 32.1 33.1 62.5 35.8 29.2	1956 2105 1937 1820 2325 2473 2060 2242 1808 2165 1958 1692 2072	25.0 20.6 26.1 26.8 24.9 29.6 29.4 29.5 31.0 29.5	2863 2404 2273 2907 2786 2003 2197 1173 2086 2096
Mean	27.0	2762	31.7	2047	27.2	2279
S. D.	± 1.1	± 335	± 4.7	± 229	± 3.2	± 515



TABLE VII

Adrenals - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact			DCA (Chronic)		DCA (Acute)		
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity		
	50.0 28.5 34.1 30.5 19.1 24.6 42.3 33.8 15.8 10.1 29.2 40.4 34.3 33.1 23.2 22.7	2939 5643 3497 4901 6094 3938 3719 4499 6167 5256 3676 3383 3725 3054 3483 2541	40.7 24.1 23.8 59.7 25.2 20.9 31.7 24.5 16.2 27.9 47.1 39.4 28.2 25.1 40.1 39.4 21.7	4576 4860 9709 2809 3941 6632 4513 5525 4330 7084 3619 5014 -- 3653 6897 6715 3471	83.1 41.2 34.8 28.9 22.3 26.5 60.4 57.1 23.2 42.8	3837 4286 4632 4235 5061 4771 3874 4946 4049 --	51.1 26.7 70.1 33.4 58.1 44.0 19.5 21.2 20.3 15.2	3906 6860 3058 5911 3086 3696 5561 6913 5964 6169
Mean			30.9	5184	41.8	4410	38.2	5112
S. D.			± 11.2	± 1735	± 20.9	± 460	± 19.0	± 1516





TABLE VIII

Ventral Prostate - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				DCA (Chronic)		DCA (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	14.0 11.7 11.9 17.9 14.4 13.1 25.9 15.4 18.6 16.6 17.8 20.4 14.8 11.3 10.5	5780 6400 6581 7034 5562 7167 5405 6951 6102 5991 5238 3869 5381 5209 4194	25.9 25.5 21.0 27.8 25.7 26.7 15.5 13.3 16.8 23.8 17.1 14.1 14.9	6471 6745 6758 4022 6686 7286 5750 6936 6202 5456 6803 6915 8181	21.1 11.8 10.6 9.7 36.3 22.2 19.7 17.1 22.6 33.0	5145 5651 9170 7178 1285 2296 2504 3967 1602 1890	11.7 18.0 24.9 12.6 11.9 15.3 17.4 20.7 16.7 18.0	11209 7864 6437 12422 5797 8177 8714 5318 7219 9005
Mean			20.2	6428	20.4	4068	16.7	8216
S. D.			± 5.8	± 998	± 8.9	± 2658	± 4.1	± 2240



TABLE IX

## Ventral Prostate - Adrenalectomized Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		DCA (Chronic)		DCA (Acute)		
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	
	17.8 19.4 17.7 15.6 17.8 15.5 21.8 15.7 16.9	6810 6585 6100 5293 5500 4542 6071 7268 5314	16.6 19.8 15.5 17.8 18.6 22.9 14.4 21.3 15.3 22.6 15.7 21.7	5955 4740 5473 4793 4676 -- 5650 4510 5626 4604 5744 4628	19.8 15.1 15.8 11.4 28.4 18.7 24.4 14.3 13.6 23.7	5610 7069 4695 5857 3022 -- 8016 5023 4952 7945	
Mean	17.6	5954	18.5	5127	19.0	6484	
S. D.	± 2.2	± 848	± 3.3	± 623	± 5.8	± 1632	



TABLE X

## Dorsolateral Prostate - Intact Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				DCA (Chronic)		DCA (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	122.9 76.2 99.5 97.8 95.0 68.5 66.3 119.2 58.6 71.7 57.6 61.7 151.0 200.0 130.5	5748 5904 3892 3251 5496 6023 4036 4128 5113 4403 6410 4544 3207 4199 2142	93.0 83.1 90.0 94.8 101.2 106.0 118.8 90.9 117.4 113.6 122.5 150.3 208.1 90.5 76.7	5582 4717 2794 3488 3195 3795 2701 4906 3412 3564 4929 5014 4124 6135 6426	194.3 68.5 151.5 60.6 70.7 138.7 78.1 99.7 120.3 154.7	4601 5395 4870 5456 4978 1878 1368 1858 3642 1333	132.8 74.1 95.6 87.1 195.6 86.4 91.4 148.1 132.4 75.7	4449 5856 4212 2781 4832 4674 3506 3416 5518 5428
Mean			112.7	4222	113.4	3538	111.9	4467
S. D.			±38.4	±1173	±43.0	±1594	±45.6	±1001





TABLE XI

## Dorsolateral Prostate - Adrenalectomized Animals

Effect of Desoxycorticosterone Acetate on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		DCA (Chronic)		DCA (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	89.4 78.1 90.6 96.9 132.2 175.9 81.2 88.8 112.1	4389 4464 4763 4706 4683 3357 4038 5172 4021	90.6 128.7 86.4 124.9 99.0 112.7 99.9 80.3 131.9 165.5 167.4 136.4	3905 4087 3205 4086 3988 4445 2676 3324 2807 2702 1141 4519	96.6 140.0 102.7 114.8 88.1 101.1 130.4 113.3 95.2 126.6	3044 2935 3018 2018 4879 3406 6622 7017 2584 5650
Mean	105.0	4399	130.5	3407	110.9	4117
S. D.	± 31.4	± 532	± 49.7	± 951	± 17.4	± 1849



## APPENDIX B



TABLE I

Serum - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				ACTH (Chronic)				ACTH (Acute)			
	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity
	328	0.5	2671	88	308	1.3	3174	98	267	1.5	3595	96
	320	1.8	4115	132	277	0.9	2699	75	262	1.0	3878	102
	328	0.6	3858	127	288	0.8	3125	90	255	1.4	3951	101
	335	0.7	3682	123	293	0.7	2936	86	288	0.9	3819	110
	330	0.9	3655	121	245	0.9	2928	72	257	0.6	2609	67
	335	1.1	3213	108	275	0.8	3550	98	262	1.0	3181	83
	277	1.5	2881	80	275	0.7	2710	75	316	1.0	3267	103
	257	1.0	2623	67	316	0.7	2856	90	282	0.7	2991	84
	252	0.9	3257	82	304	0.8	2741	83	284	0.6	2492	71
	243	0.7	3335	81	291	1.1	2750	80	296	0.4	2966	88
	236	0.7	4034	95	326	0.7	2930	96	275	0.5	2662	73
	225	1.5	4556	103	267	1.6	--	--	260	0.6	2967	77
	233	0.9	2561	60	315	1.3	--	--	275	--	--	--
	268	1.4	3763	101	282	--	--	--				
Mean	283	1.0	3443	97.7	290	1.0	3240	94.3	275	0.9	3198	87.9
S. D.		$\pm 0.39$	$\pm 613$	$\pm 22.6$		$\pm 0.3$	$\pm 401$	$\pm 13.1$		$\pm 0.39$	$\pm 510$	$\pm 14.2$



TABLE II

Serum - Adrenalectomized Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact				ACTH (Chronic)				ACTH (Acute)			
	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g./gm.}$	Specific Activity	Corrected Specific Activity
	267	0.8	1884	50	327	0.9	4196	137	295	0.7	9560	282
	283	0.5	1831	52	324	0.6	4354	141	312	0.5	6752	211
	295	0.3	1838	54	328	0.6	3276	109	329	0.6	7487	246
	286	0.4	2172	62	345	0.6	4171	144	323	1.1	4862	157
	294	0.5	2483	73	317	0.5	5503	174	368	1.0	7443	274
	295	0.4	2646	78	230	1.4	5354	123	363	1.1	6670	242
	316	0.4	2415	76	234	1.2	5430	127	350	1.3	6001	210
	321	0.6	2369	76	244	1.7	5857	143	313	0.6	6563	205
	241	0.8	2297	55	227	1.4	6203	141	371	1.7	4647	172
	311	0.5	1655	52	250	1.2	3356	83	165	0.8	5191	86
	281	0.5	1580	44	238	0.7	3168	75	177	1.4	4785	85
	315	0.5	1504	47	245	1.6	--	--	305	2.6	--	--
	304	0.6	2912	89	259	1.3	--	--	178	1.8	--	--
	254	0.6	3135	80	293	0.7	--	--	176	1.1	--	--
	270	0.6	2511	68								
Mean	289	0.5	2216	63.7	276	1.0	4624	127.0	287	1.16	6360	197.3
S. D.		$\pm 0.14$	$\pm 490$	$\pm 14.0$		$\pm 0.43$	$\pm 1154$	$\pm 31.5$		$\pm 0.57$	$\pm 1485$	$\pm 67.1$





TABLE III

Cells - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	5.3 7.7 6.7 4.9 5.3 10.8 8.6 8.4 8.4 7.8 7.1 8.3 5.4 11.6	1940 -- 1717 1297 1580 1661 1912 1635 1774 1437 2009 1622 1639 --	6.5 7.3 6.6 -- 8.2 8.6 7.8 8.5 8.0 9.2 8.2 8.0 6.9 7.2	1999 2280 1750 -- 2003 2071 1941 2344 1619 1612 1445 1988 1999 1948	8.4 8.4 8.1 6.6 7.2 5.6 5.9 6.4 5.3 4.3 5.8 5.0 5.0	1603 2072 1685 1702 2300 1905 1914 1918 1562 1614 1834 1797 1621
Mean	7.6	1685	7.7	1923	6.3	1810
S. D.	± 2.1	± 205	± 0.82	± 260	± 1.36	± 213



TABLE IV

Cells - Adrenalectomized Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	8.3 9.0 7.3 7.3 5.6 6.6 7.9 4.7 8.4 7.5 8.4 6.2 8.6 8.2 7.2	2163 2364 1970 1736 2181 2154 3029 2116 1739 1756 1477 1642 1516 1940 1966	8.2 7.8 7.3 6.8 6.5 6.3 6.1 10.3 9.7 7.5 10.6 7.0 10.0	1857 1638 2750 4036 2579 2930 2697 2272 1972 1912 1690 1690 1291	8.9 8.9 8.3 8.5 5.9 5.2 10.5 5.4 7.8 6.5 10.7 9.5 9.4 8.4	3066 3204 2550 3030 2698 2564 2654 2309 2466 2793 3195 3640 2720 3104
Mean	7.5	1983	8.0	2255	8.1	2857
S. D.	± 1.2	± 395	± 1.6	± 735	± 1.8	± 362



TABLE V

Liver - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	36.4 43.9 43.3 51.4 53.0 53.5 48.1 57.8 52.2 55.2 56.6 45.9 45.3 51.7	3021 2944 3323 3356 3037 2531 3478 2970 2984 3098 3636 3086 2962 2903	44.8 41.4 42.1 -- -- 42.5 46.7 42.4 55.3 45.3 51.1 53.4 52.0 48.1 37.8 45.2	3097 3522 3185 -- -- 3180 2865 3797 3730 2777 3147 2600 3027 3997 3404 2993	51.7 48.1 54.3 53.2 51.3 49.5 46.7 48.3 41.5 51.5 45.0 54.1 40.9 41.8	4339 4897 4843 3839 4324 4833 4455 4342 3539 3783 3729 4003 3777 4038
Mean	49.6	3095	46.3	3237	48.4	4196
S.D.	± 6.0	± 276	± 5.1	± 405	± 4.6	± 450





TABLE VI

Liver - Adrenalectomized Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	58.0 44.6 44.1 47.3 46.4 46.1 42.5 47.9 59.6 43.6 48.1 41.0 40.7 41.3 42.4	4370 2514 2750 2770 3939 1806 4038 3452 5956 3311 3353 2365 3349 2047 4407	69.7 56.5 55.4 44.1 46.5 51.8 48.7 45.0 43.3 47.2 50.3 56.6 59.6 71.0	3334 5322 6183 4665 5551 4658 2190 3548 2326 2997 3322 3946 -- 4805	63.6 50.5 75.9 69.2 61.2 53.5 54.8 59.1 57.7 56.9 57.0 70.3 47.0 75.5	5192 4764 5983 5821 5798 5631 4995 5501 5662 5187 4644 5717 4999 6208
Mean	46.2	3362	53.2	4065	60.9	5436
S.D.	± 5.7	± 1076	± 8.8	± 1149	± 8.9	± 475



TABLE VII

Testis - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	29.0 30.4 29.6 27.2 29.7 28.1 27.3 30.7 32.7 30.0 30.1 32.9 29.4 28.0	949 1127 1149 1046 1103 1175 1269 1075 1056 1163 1155 1209 1240 1031	28.7 29.1 32.9 26.8 28.0 28.6 27.0 26.5 26.5 26.6 28.4 28.7 35.3 28.3	990 1159 1077 1140 1026 1189 1060 941 673 975 997 1049 656 1037	29.7 30.3 30.8 30.2 28.4 31.5 30.4 30.1 31.1 29.3 28.8 31.6 30.8 27.7	1080 1203 1009 1070 1449 1260 1219 1040 1106 1142 1221 1079 1079 981
Mean	29.7	1125	28.7	998	30.1	1138
S. D.	± 1.6	± 88	± 2.5	± 158	± 1.1	± 123



TABLE VIII

Testis - Adrenalectomized Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	30.6 31.0 30.3 29.7 30.3 30.6 30.2 30.1 31.1 30.6 29.3 31.1 29.7 28.9 31.4	1176 1066 1332 1149 1036 1061 1048 1012 1155 1204 1189 1051 1037 1253 1275	28.0 29.9 28.4 27.7 29.8 25.7 30.4 27.5 30.0 29.7 29.9 30.5 32.5 31.9	1260 1740 2179 1357 1736 1717 1639 1577 1112 1712 1253 1496 1645 1549	29.7 30.6 29.9 28.9 28.2 30.3 28.4 30.7 29.2 40.6 29.8 23.6 30.3 29.4	1735 1418 1793 1776 1746 1930 1419 1680 1588 2590 2058 1670 2123 2491
Mean	30.3	1136	29.4	1569	30.0	1858
S. D.	± 0.7	± 32	± 1.8	± 269	± 3.5	± 331



TABLE IX

Adrenals - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	25.2 20.9 31.7 24.5 16.2 27.9 28.9 26.8	2189 3684 2507 3069 2406 3936 3101 2972	18.9 15.9 26.6 23.8 25.7 19.7 27.6 20.6 29.0 18.6 27.0 42.1 21.3 28.3	4968 3792 2785 3649 2586 5335 3425 3696 2596 3348 2789 2171 4248 2459	42.5 33.4 44.6 31.0 33.5 30.5 35.8 42.7 41.6 29.9 38.3 42.8 31.3 37.6	2937 3649 2208 2894 3233 3302 3000 2335 2149 2747 2293 2177 2744 2232
Mean	25.3	2983	24.7	3418	36.8	2707
S. D.	± 4.8	± 611	± 6.5	± 948	± 5.3	± 486





TABLE X

## Ventral Prostate - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	15.5 13.3 16.8 23.8 17.1 17.5 22.1 20.0 19.6 19.7 19.8 22.8 19.3 14.9	3142 3790 3389 2981 3599 3830 3835 3512 2981 3594 3595 3635 3202 --	14.1 14.6 16.0 16.9 16.2 18.0 17.6 16.3 14.3 13.9 17.0 18.7 18.4 15.0	4086 4250 3610 3858 3625 3967 3882 3143 3295 3747 3518 4170 3715 3946	16.8 18.4 16.4 16.8 15.2 16.6 20.0 14.7 16.0 20.3 14.0 18.2 16.9	3308 3808 3427 3456 3833 3715 3901 3922 3385 3537 3816 3467 3217
Mean	18.7	3468	16.2	3773	17.0	3599
S. D.	± 3.0	± 304	± 1.8	± 318	± 1.9	± 242

1. 關於「國民教育」

2. 關於「社會教育」

3. 關於「職業教育」

4. 關於「師範教育」

5. 關於「體育」

6. 關於「音樂教育」

TABLE XI

Ventral Prostate - Adrenalectomized Animals  
Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	18.3 13.8 18.6 16.5 18.0 14.0 17.7 17.2 15.8 17.1 14.5 20.8 15.8	2795 3407 3358 3144 3257 3004 2876 3051 2743 2690 3283 2536 3921	17.2 15.1 13.0 15.0 13.7 17.1 14.2 15.7 13.5 16.9 19.1 15.4 23.4 18.0	3839 4417 6469 4255 5101 4556 5925 4678 6387 4251 3724 3546 5401 4857	13.2 15.8 13.7 24.2 13.8 15.3 16.5 17.9 30.0 22.9 12.6 12.7 26.4 26.2	6780 5417 5951 4707 6016 5622 4572 5346 5047 4220 -- 5657 5158 5212
Mean	16.8	2671	16.2	4815	18.7	5362
S. D.	± 2.0	± 439	± 2.9	± 941	± 6.0	± 757



TABLE XII

Dorsolateral Prostate - Intact Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact		ACTH (Chronic)		ACTH (Acute)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	117.4 113.6 122.5 150.3 208.1 90.5 123.2 86.8 128.5 108.1 113.6 80.4 167.8	1706 1782 2464 2507 2062 3068 2860 2719 1998 2364 2284 2403 --	193.8 105.4 154.5 133.9 124.2 110.2 103.6 78.0 89.7 143.9 114.8 115.8 103.9	1745 2633 2573 2461 2409 3202 2553 2745 2386 2270 2066 2712 2793	189.4 88.7 111.9 185.8 80.5 131.4 93.4 122.2 130.5 141.3 127.1 109.8 125.7	2137 3594 1985 1914 2182 2541 2627 2765 1915 1971 2329 1942 2145
Mean	123.9	2351	120.9	2511	126.0	2311
S. D.	± 34.9	± 416	± 30.2	± 259	± 32.8	± 526





TABLE XIII

## Dorsolateral Prostate - Adrenalectomized Animals

Effect of ACTH on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Adrenalectomized		ACTH (Chronic)		ACTH (Acute)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
	85.8 87.8 194.0 95.0 175.9 223.2 148.1 118.2 82.7 146.8 96.6 158.2 176.3 129.5 135.8	2342 2395 1645 2122 1367 1362 2414 1603 1888 1491 1887 1644 1442 1941 1795	141.9 170.8 142.8 215.0 143.2 146.4 129.5 103.7 107.4 134.3 97.5 71.7 107.3 99.4	1844 3481 4385 2078 2606 2200 3918 3920 3516 3491 2133 2633 2896 3258	219.2 260.9 256.1 164.3 232.6 266.5 213.0 117.5 77.6 77.6 130.8 88.0 94.3	4037 2289 3047 3601 3899 2333 3870 4657 4112 4887 3139 4782 4389
Mean	136.9	1823	129.1	3026	169.0	3773
S. D.	± 43.2	± 332	± 36.1	± 798	± 74.7	± 859



## APPENDIX C



TABLE I

Serum - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*			Growth Hormone (Chronic)				
	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	
					355	1.6	2240	80
					341	1.6	2830	97
					362	1.4	2376	86
					340	2.1	3530	120
					340	1.4	2382	81
					372	1.9	2885	107
					336	1.1	2617	88
					378	1.6	2345	89
					318	1.3	2611	83
					343	1.0	2139	73
					333	0.9	3414	114
					322	1.1	3414	110
					389	1.0	2223	88
					375	1.9	2370	89
					330	1.6	2727	90
					346	1.3	--	--
Mean	283	1.0	3443	97.7	349	1.4	2674	93.0
S. D.		$\pm 0.39$	$\pm 613$	$\pm 22.6$		$\pm 0.37$	$\pm 460$	$\pm 13.7$

\*Values for intact animals obtained from Appendix B, Table I.



TABLE II

Serum - Hypophysectomized Animals  
Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

Hypophysectomized				Growth Hormone (Chronic)			
Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity	Animal Wt. (gm.)	Zn $\mu\text{g.}/\text{gm.}$	Specific Activity	Corrected Specific Activity
181	1.2	4390	80	258	0.7	2506	65
197	1.9	3891	77	249	1.6	2468	61
181	0.5	3050	55	228	1.1	1915	44
207	1.6	2975	62	218	2.4	3126	68
190	1.0	3643	69	205	1.1	2619	54
158	1.2	2794	44	211	1.3	3643	77
164	0.6	4255	70	221	1.4	3291	73
165	0.7	2310	38	200	1.4	2558	51
185	0.9	1900	35	207	1.1	3233	67
159	0.9	2533	40	204	0.8	2160	44
161	0.6	3355	54	213	0.9	2179	46
161	1.4	2183	35	214	1.1	2951	63
147	1.3	2857	42	247	0.8	--	--
162	1.3	--	--	186	0.7	--	--
				233	0.7	--	--
				223	1.2	--	--
Mean	1.1	3087	53.9	220	1.1	2720	59.4
S. D.	$\pm 0.41$	$\pm 784$	$\pm 16.3$		$\pm 0.44$	$\pm 528$	$\pm 11.4$





TABLE III

Cells - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
			5.3 6.7 10.2 6.5 4.8 7.8 6.9 9.3 7.0 6.1 7.4 8.8 7.9 6.6 6.9 6.3	1986 1864 1718 1517 1672 1506 1693 1757 1396 1414 960 1201 1402 1904 1474 1716
Mean	7.6	1685	7.2	1574
S. D.	$\pm 2.1$	$\pm 205$	$\pm 1.4$	$\pm 269$

\*Values for intact animals obtained from Appendix B, Table III.



TABLE IV

## Cells - Hypophysectomized Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Hypophysectomized		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	8.3 7.7 10.1 7.2 8.3 8.0 11.4 9.4 10.7 10.4 12.7 10.0 9.4 8.7	2377 2527 1707 1931 2530 2215 2338 1759 2088 2773 2224 1847 -- --	6.6 7.8 7.5 10.8 10.4 11.0 9.6 11.5 9.8 10.0 11.1 10.3 5.9 5.1 8.2 8.4	1443 1592 1763 1644 1940 1614 2724 1327 2029 1769 1828 1743 1504 1656 1577 1883
Mean	9.5	2193	9.0	1752
S. D.	± 1.6	± 336	± 1.9	± 356



TABLE V

Liver - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
			41.8 41.1 41.8 40.2 51.3 45.5 44.5 53.6 44.9 45.1 50.4 57.7 46.7 47.7	2908 3079 3161 3538 2882 2648 3315 2690 3271 3253 3186 3149 2801 2903
Mean	49.6	3095	46.6	3058
S. D.	± 6.0	± 276	± 5.1	± 256

\*Values for intact animals obtained from Appendix B, Table V.





TABLE VI

Liver - Hypophysectomized Animals  
Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Hypophysectomized		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	49.3 49.2 56.1 46.4 92.2 54.2 58.7 57.6 65.0 66.6 48.9 62.0 57.9 52.9	5262 4891 4441 3984 5451 5009 4750 3908 4554 4674 4848 6319 4070 --	53.2 55.2 48.0 48.9 42.0 57.2 46.2 50.8 51.2 37.4 37.9 45.5 53.2 60.2 50.8	3044 3392 3573 4144 4147 5017 2679 4317 3681 3506 3739 3705 3153 2856 3317
Mean	58.4	4782	49.2	3618
S. D.	$\pm$ 11.5	$\pm$ 658	$\pm$ 6.6	$\pm$ 609



TABLE VII

Testis - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (Chronic)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
			29.9 32.4 31.2 30.8 30.3 30.5 30.4 30.1 30.9 30.9 31.2 31.9 31.8 31.7 34.9 31.4	974 1000 967 975 1036 872 1036 910 1022 784 1067 1024 807 930 861 962
Mean	29.7	1125	31.3	952
S. D.	± 1.6	± 88	± 1.2	± 85

\*Values for intact animals obtained from Appendix B, Table VII.



TABLE VIII

Testis - Hypophysectomized Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Hypophysectomized		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	26.4 25.5 28.7 23.6 25.1 26.9 25.2 25.6 27.2 24.0 27.8 23.0 22.9 25.4	1551 1904 1723 1715 1515 1479 1780 1976 1380 1848 989 1380 -- --	25.6 22.9 22.1 25.0 26.6 22.8 25.0 26.9 24.9 24.0 24.8 26.0 28.5 24.9 23.2 22.5	992 1414 1493 778 1675 1941 1454 1054 1656 1784 1222 1513 1293 1702 1570 1505
Mean	25.5	1603	24.7	1440
S. D.	± 1.8	± 279	± 1.8	± 309



TABLE IX

Adrenals - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
			-- 35.4 28.6 22.2 29.0 27.6 # 25.8 # 24.5 # 29.1 37.2	-- 2112 2760 2685 2879 2626 # 3440 # 2452 # -- 2712
Mean	25.3	2983	28.8	2708
S. D.	± 4.8	± 611	± 4.8	± 119

\*Values for intact animals obtained from Appendix B, Table IX.

# A pooled sample (three animals).





TABLE X

## Adrenals - Hypophysectomized Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

Hypophysectomized		Growth Hormone (Chronic)	
Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
33.6	3548	65.4	1253
37.7 #	3213 #	50.6	1127
36.5 #	2900 #	74.0	1007
62.5 #	2206 #	51.9 #	2030 #
54.6 #	2347 #	55.5 #	1720 #
		51.0 #	--
		58.7 #	--
Mean	2843	58.2	1427
S. D.	± 12.8	± 8.7	± 432

# A pooled sample (three animals).

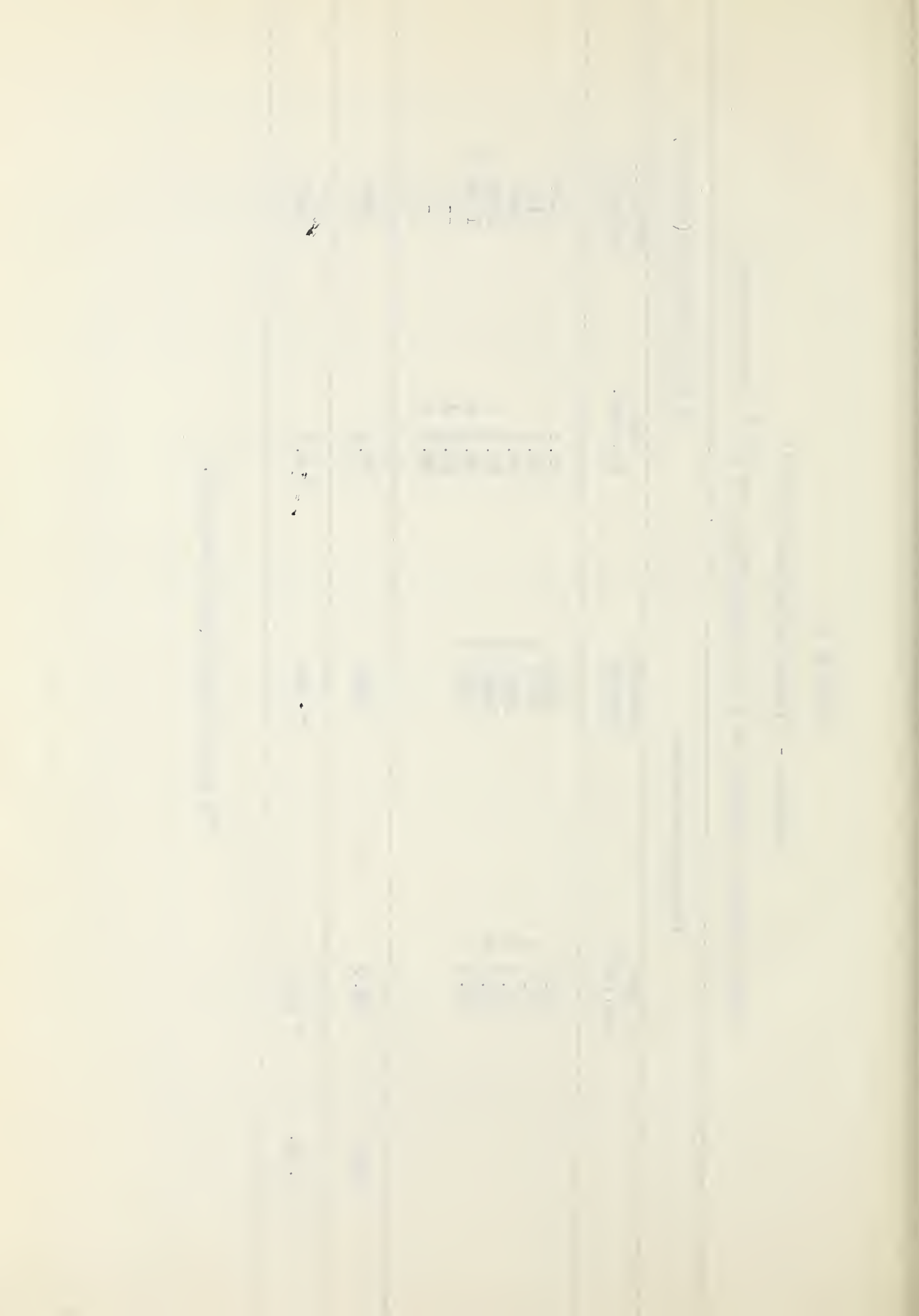


TABLE XI

Ventral Prostate - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (Chronic)	
	Zn µg./gm.	Specific Activity	Zn µg./gm.	Specific Activity
			16.5 16.3 15.4 23.8 11.4 17.4 26.2 15.8 18.9 11.6 13.2 15.6 18.4 16.2 20.8 14.0	2561 3346 3626 2615 3354 2591 1889 3644 2810 3439 2959 3086 3403 2879 2679 3517
Mean	18.7	3468	17.0	3025
S. D.	$\pm$ 3.0	$\pm$ 304	$\pm$ 4.5	$\pm$ 515

\*Values for intact animals obtained from Appendix B, Table X.



TABLE XII

Ventral Prostate - Hypophysectomized Animals  
Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

Hypophysectomized			Growth Hormone (Chronic)	
Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity	
49.8	2379	54.7	1954	
70.4	1649	55.2	1991	
60.2 #	1416 #	99.5	968	
60.1 #	1646 #	40.0	869	
44.1 #	1406 #	47.6 #	1452 #	
34.5 #	2372 #	53.7 #	555 #	
		38.4 #	1552 #	
		50.6 #	1044 #	
Mean	1811	54.1	1298	
S. D.	± 14.1	± 18.1	± 596	

# A pooled sample (three animals)





TABLE XIII

## Dorsolateral Prostate - Intact Animals

Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Intact*		Growth Hormone (chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
			121.1 215.6 157.6 156.9 144.8 157.7 131.0 137.8 142.2 123.0 239.6 163.5 138.3 203.0 183.9 140.1	1821 1490 1136 1684 1718 1573 2345 1853 1516 1773 1756 1899 1685 1243 1680 --
Mean	123.9	2351	159.8	1678
S. D.	± 34.9	± 416	± 35.1	± 294

\*Values for intact animals obtained from Appendix B, Table XII.



TABLE XIV

Dorsolateral Prostate - Hypophysectomized Animals  
Effect of Growth Hormone on Zinc Concentration and Zn<sup>65</sup> Incorporation

	Hypophysectomized		Growth Hormone (Chronic)	
	Zn μg./gm.	Specific Activity	Zn μg./gm.	Specific Activity
	36.7 39.1 44.7 41.6 46.8 70.8 103.8 102.0 61.5 66.9 73.1 47.0 75.3 47.1	2443 2664 2110 2003 1857 1431 1233 1386 1743 1970 1634 2417 2089 2502	46.6 30.3 61.7 48.1 55.0 48.1 76.5 63.0 57.0 51.0 40.7 72.0 75.1 40.2 64.3 62.9	2322 2849 1667 2306 1879 2177 1231 1022 1965 2289 2356 1317 1174 2257 1956 2142
Mean	61.2	1963	55.8	1932
S. D.	± 23.2	± 512	± 13.7	± 544















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